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Poly(*N*-isopropylacrylamide-co-acrylamide) cross-linked thermoresponsive microspheres obtained from preformed polymers: Influence of the physico-chemical characteristics of drugs on their release profiles

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Abstract

Poly(*N*-isopropylacrylamide-co-acrylamide) copolymer was synthesized as an interesting thermoresponsive material possessing a phase transition temperature of around 36 °C in phosphate buffer, pH 7.4 (PB); the concentration was 10%, w/v. The copolymer maintains a sharp phase transition at a relatively high percentage of acrylamide. The lower critical solution temperature (LCST) of the copolymer is influenced by the concentration of copolymer solution in PB. The copolymer was transformed in thermoresponsive microspheres by chemical cross-linking of amide groups with glutaraldehyde. The key factors for the successful preparation of microspheres are the use of a concentrated polymer solution, a temperature (38 °C) that is high enough but lower than LCST, and a long reaction time (48 h). The microspheres were characterized by optical and scanning electron microscopy, swelling/deswelling kinetics, swelling degree, and PB retention at different temperatures. Finally, the influence of hydrophilicity/hydrophobicity and the molecular weight of the drugs (propranolol, lidocaine, vitamin B_{12}) on their release profile from thermoresponsive microspheres were examined. Above LCST the hydrogel matrix is in the dehydrated state and hydrophobic interactions between the hydrophobic drugs and the polymer occur, modulating the release rate of the drugs. For hydrophilic drugs, the release rate is modulated mainly by the steric interaction between the drug molecule and the matrix.

Keywords: Intelligent microspheres; pH/thermoresponsive copolymers; Equilibrium swelling ratio; Lower critical solution temperature; Drug delivery

1. Introduction

Thermally responsive drug delivery systems have attracted ever-increasing attention because they can control the release of drug in response to changes in body temperature and therefore act as self-regulating systems [1–4]. Poly(*N*-isopropylacrylamide) (PNIPAAm) is the most popular polymer among the thermoresponsive polymers since it exhibits a sharp phase transition close to 32 °C [5,6]. 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. The biomedical and biological applications of such gels usually involve the chemical modification of poly(NIPAAm). These modifications are usually performed to introduce

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functional groups that can increase the LCST towards body temperature [7,8], to improve the mechanical properties [9] or to interact with certain drugs [10]. However, copolymerization of NIPAAm with acrylate-type comonomers usually leads to gels possessing relatively weak thermosensitivity [11]. Therefore, the comonomer needs to be chosen carefully to preserve the thermosensitivity of the gel structure. Most of the studies concerning the applications of thermoresponsive hydrogels have focused on the use of devices in the form of discs or slabs [12–14]; few papers have dealt with the preparation and characterization of thermoresponsive microspheres. The majority of microspheres are prepared from monomers by suspension polymerization [15,16]. Thermoresponsive microspheres from preformed polymers are prepared by dropping a polymer solution into a liquid at a temperature above the LCST [17–19]. These microspheres are not stable or easy to handle, and have a reduced number of biomedical applications. The most studied drug used as a model for pulsatile on–off drug release from thermoresponsive hydrogels is the hydrophobic indomethacin [20,21].

The main objectives of this paper were the preparation of stable thermoresponsive microspheres from performed polymers and the study of the influence of physico-chemical characteristics of drugs on their release profile.

Here, the poly(NIPAAm-co-AAm) copolymer was prepared as a thermoresponsive polymer with it's LCST tailored towards body temperature. This copolymer was transformed into thermoresponsive stable microspheres by an original approach that assumes the cross-linking of the amide group of acrylamide with glutaraldehyde under particular conditions (long reaction time, temperature slightly below the LCST, concentrated polymer solution). The microspheres were characterized by optical and scanning electron microscopy in the dried, swollen and shrunken state, and the degree of swelling and rate of swelling/deswelling were determined. Finally, the influences of drug hydrophilicity/hydrophobicity and molecular weight on the release profiles were examined.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAAm) (from Aldrich Chemical Corp., Milwaukee, WI, USA) was recrystallized with hexane. Acrylamide (AAm), glutaraldehyde (GA) aqueous solution (25% W/V) and *N*,*N*′-azobisisobutyronitrile (AIBN) were supplied from Fluka AG (Buchs, Switzerland). AIBN was purified in methanol before use. Blue dextran (BD) was provided from Pharmacia (Uppsala, Sweden). 1,4-Dioxane, from Fluka AG, was purified by refluxing. Light mineral oil ($d = 0.84\ g\ ml^{-1}$) was supplied by Sigma Chemical Co. (St. Louis, MO, USA) and the model drugs used for loading and release studies were provided from Iassy Pharm S.A. (Iassy, Romania). All chemicals were of the highest analytical grade.

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

Synthesis of linear poly(NIPAAm-co-AAm) was carried out by free radical copolymerization in 1,4-dioxane with AIBN as initiator. Typically, 1.13 g of NIPAAm, 0.142 g of AAm and 0.010 g of AIBN were solubilized in 6 ml of 1,4-dioxane. Dried nitrogen was bubbled through the solution for 30 min prior to polymerization. The reaction mixture was allowed to react at 70 °C until the gel point was reached (about 4 h). Then 6 ml of dioxane was added and the reaction was allowed to continue for 16 h. The polymer was precipitated into diethyl ether and dried under vacuum. Finally, the copolymer was solubilized in distilled water, dialysed for 5 days at 20 °C and recovered by freeze-drying.

2.3. Determination of molecular weight

The number-average (M_n) and weight-average (M_w) molecular weights of poly(NIPAAm-co-AAm) were determined by gel permeation chromatography using a GPC-PL-EMD 950 instrument (Polymer Laboratories, Shropshire, UK) in dimethylformamide at 120 °C at a flow rate of 0.7 ml min^{-1}. Calibration was carried out with monodisperse polystyrene standards.

2.4. Copolymer composition

Copolymer composition was determined by ^{13}C nuclear magnetic resonance (NMR) analysis. ^{13}C NMR spectra of poly(NIPAAm-co-AAm) were recorded in deuterated dimethylsulfoxide solution of copolymers on a Varian Mercury Plus 400/Varian VXR 200 spectrometer operating at 200 MHz frequency, using a relaxation delay of 30 s to accumulate 7648 transits. The mole fraction of AAm in poly(NIPAAm-co-AAm) was calculated from the area of the peak at 176.60 ppm, due to the carbonyl carbon of AAm, and from the area of the peak at 173.16 ppm, attributed to the carbonyl carbon of NIPAAm.

2.5. LCST determination

LCST was determined from the dependence of absorbance at 450 nm on temperature using an ultraviolet–visible light (UV–Vis) spectrophotometer (Specord 200, Analytic Jena, Germany) coupled with a temperature controller. The polymer solution was prepared under standard acidic solution (pH 1.2, 64 mM HCl + 50 mM KCl), and standard phosphate buffer (pH = 7.4, 50 mM Na_2H-PO_4 + NaOH; PB). The heating rate was 2 °C every 10 min and 0.2 °C in the vicinity of the cloud point (CP). The CP was defined as the temperature at 10% absorbance in the curve of the normalized absorbance vs. temperature.

2.6. Microsphere preparation

Typically, 600 mg of copolymer was solubilized in 3 ml of distilled water at 4 °C. Then 0.2 ml of 0.5 M H_2SO_4

ID	Title	Pages
2376	Poly(N-isopropylacrylamide-co-acrylamide) cross-linked thermoresponsive microspheres obtained from preformed polymers: Influence of the physico-chemical characteristics of drugs on their release profiles	11