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Smart nanoparticles based on pullulan-g-poly(N-isopropylacrylamide) for controlled delivery of indomethacin

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ABSTRACT

Double hydrophilic thermo-responsive pullulan-g-poly(N-isopropylacrylamide) (P-g-pNIPAM) copolymers with two different molecular weight of thermosensitive grafts were synthesized and used for preparation of indomethacin-loaded nanoparticles by dialysis and nanoprecipitation method. The polymers form aggregates in aqueous solution at a concentration of 10 g/L, above their critical aggregation concentration (3.36 g/L) and below the lower critical solution temperature (LCST). After indomethacin loading, nanoparticles with compact and uniform structure were formed below the LCST. The effects of copolymer composition, concentration, and the feed polymer/drug ratio on the particle size, drug loading content (DLC) and entrapment efficiency (EE) were investigated. DLC increased with drug feeding, reaching a maximum value of 40% at the ratios of 1/1. Smaller particles (145 nm) with narrower size distribution were obtained from polymer with a higher molecular weight of pNIPAM grafts.

FT-IR and ^{1}H NMR spectra proved that the main driven force for the aggregation was the hydrogen bonding between indomethacin and the pNIPAM side chains of copolymer.

The indomethacin release rate from nanoparticles was influenced by temperature, because of the dissociation of the hydrogen bonds at high temperatures, the degree of drug loading, and the pH of the release media.

1. Introduction

Over the past years, polymeric micelles with core-shell morphology have been gained much attention for their potential application in the field of drug delivery [1]. Nanoparticles obtained by self-assembly of amphiphilic [2–4] or double-hydrophilic copolymers [5,6] are effective and efficient carriers for hydrophobic and poorly water-soluble drugs which can be entrapped by hydrophobic, hydrogen-bonding or electrostatic interactions. The micellization of these copolymers can be controlled by changes in the temperature, pH, ionic strength, etc. when one of the polymeric chains may undergo a phase transition from hydrophilic to hydrophobic state. Also, guest molecules that interact with the polymer segments can be used to trigger micellization [7–10].

In recent years, researchers are interested in smart drug delivery systems that are able to release the therapeutic payload on demand. Nanoscale drug delivery vehicles formulated from biocompatible and biodegradable thermoresponsive polymers represent a growing approach to lipophilic drug delivery.

Recently, due to its special property, poly(N-isopropylacrylamide) (pNIPAM) has been used in many copolymers to design stimuli-responsive polymeric micelles for drug delivery purposes [11–13]. pNIPAM exhibits a reversible phase transition in aqueous solution at about 32 °C, which is known to be the lower critical solution temperature (LCST). The micelles can be constructed when the temperature is raised above the LCST of the block copolymers containing pNIPAM, where pNIPAM forms the hydrophobic core. The block copolymers compose the so-called thermosensitive micelle-forming polymers.

Newly, pNIPAM was coupled into amphiphilic block copolymers with poly(ethylene glycol) [14], poly[N-(2-hydroxypropyl) methacrylamide] [15] as hydrophilic blocks and graft copolymers with polyphosphazenes [16,17] or dextran [18] as backbone and pNIPAM as side groups. These double hydrophilic graft copolymers

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formed nanoparticles below the LCST triggered by hydrogen bonding between the copolymers and a hydrophobic drug.

In order to develop new nanoparticulate thermoresponsive carrier with good entrapment efficiency for lipophilic drugs, we considered the possibility to use pullulan-grafted-pNIPAM copolymers for production of indomethacin (IND) loaded nanoparticles. IND was used as model drug to incorporate into nano-aggregates due to its hydrophobic character. In particular, this paper describes the preparation of polymeric nanoparticles under LCST of grafted copolymers in order to evaluate their potential and efficiency as drug carriers.

The IND-loaded polymeric particles were prepared by different methods. The characteristics of these particles were investigated through dynamic light-scattering (DLS) and atomic force microscopy (AFM). In addition, the drug loading efficiency of incorporated IND was investigated by ultraviolet (UV) spectrophotometry. The drug release kinetics of prepared nanoparticles have also been investigated at different temperatures, below and above the LCST.

2. Experimental and methods

2.1. Materials

Pullulan (P), Mw = 200,000 g/mol, was purchased from Hayashibara Lab. Ltd. (Okoyama, Japan). N-isopropylacrylamide (NIPAM) (Aldrich Chemical Corp., Milwaukee, WI, USA) was recrystallized with hexane. Ceric ammonium nitrate (CAN) and other chemicals were purchased from Fluka AG (Buchs, Switzerland) and used without further purification.

Indomethacin (IND) was obtained from Sigma Co. (St. Louis, USA).

2.2. Synthesis and characterization of P-g-pNIPAM copolymers

2.2.1. Synthesis

P-g-pNIPAM was synthesized by free radical polymerization using CAN as initiator [19]. Thus, pullulan (1.0 g, 6.17 mmol) was dissolved in 12 mL of 0.01 M nitrate acid solution in a three-necked flask equipped with a nitrogen inlet and a reflux condenser immersed in a thermostated water bath. The solution was purged with nitrogen for 30 min and the nitrogen atmosphere was maintained throughout the polymerization period. Then, CAN (0.88 g, 1.60 mmol) solubilized in 4 mL of 0.01 M nitrate acid solution was added. After 10 min supposed to be enough to induce free radicals onto pullulan chains, corresponding amount of NIPAM (1.0 g) was added to flask, and the reaction continued for 20 h at 25 °C. The reaction was stopped by addition of 1N NaOH aqueous solution. The reaction mixture was finally poured into methanol, filtrated, washed and extracted with methanol for 24 h in order to remove pNIPAM homopolymers. The product was dried under vacuum at 60 °C.

2.2.2. Structural characterization

^{1}H NMR and FT-IR spectrometry was used to study the structure and the molar composition of graft copolymer. To obtain the molecular weight of grafted pNIPAM, P-g-pNIPAM copolymers were hydrolyzed by 72% sulfuric acid solution at room temperature for 7 h. The viscosity-average molecular weights of pNIPAM grafts were determined by viscometric measurements in THF at 27 °C using the following equation [20]:

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

2.2.3. LCST measurement

The absorbance of the P-g-pNIPAM copolymer aqueous solution was measured at a wavelength of 450 nm using an UV–vis Evolution 201 spectrophotometer (Thermo Fisher Scientific Inc., Madison, USA) coupled with a temperature controller. The absorbance change was recorded as a function of temperature from 25 °C to 40 °C. The polymer solutions (1%, w/v) were prepared in ultrapurified water. The heating rate was 1 °C every 10 min. The cloud point was defined as the temperature at 50% absorbance in the curve of the normalized absorbance versus temperature.

2.2.4. Critical aggregation concentration (CAC)

The *CAC* of the copolymers was determined by fluorescence technique. Fluorescence spectra of pyrene solubilized in the copolymer solutions were recorded with an LS 55 PerkinElmer spectrophotometer (Waltham, Massachusetts, USA) equipped with a thermostated cell holder at 25 °C. The stock solution of pyrene ($\sim 2.0 \times 10^{-6}$ M) was prepared in double-distilled water. Experiments were performed by solving the polymer in the pyrene solution for 24 h before the measurements.

The excitation wavelength was 335 nm and slits set at 8 and 2.5. The fluorescence spectra were recorded from 350 to 600 nm. The ratio I_1/I_3 of the fluorescence intensities of the first (373 nm) and third vibronic peaks (384 nm) was then calculated, which provides a measure for the polarity of the microenvironment of pyrene at binding sites in hydrophobic microdomains.

2.3. Preparation of IND-loaded nanoparticles

P-g-pNIPAM nanoparticles containing IND were prepared by both the dialysis method [21] and the nanoprecipitation technique [22] at room temperature. IND was used as model drug with hydrophobic nature.

In the dialysis method, P-g-pNIPAM copolymer was dissolved in 5 mL of dimethylformamide followed by the addition of IND with various weight ratios to polymer (0.5/1 and 1/1) and stirred at room temperature. To form IND-loaded nanoparticles and remove free IND, the solution was dialyzed for 3 days against 1000 mL of ultrapure water using regenerated cellulose dialysis membranes (molecular weight cut-off of 12,000 (Sigma-Aldrich, Germany)). During the first day, water was exchanged every 4 h and then each day. The nanoaggregates solution was sonicated using ultrasonic bath (Cole-Parmer, Illinois, USA), and then centrifuged (Hettich Universal 320R, Tuttlingen, Germany) to remove unloaded IND and aggregated particles. The supernatant was analyzed by DLS or freeze dried for 24 h for further studies. Each experiment was performed in triplicate.

In the nanoprecipitation method, IND and P-g-pNIPAM copolymer in various weight ratios ($0.33/1 \div 2/1$) were dissolved in 5 mL of dimethylformamide. Then, the solution was slowly dropped into 15 mL of distilled water under magnetic stirring at 500 rpm. The obtained opalescent solution was allowed to stir for 30 min at room temperature, then it was dialyzed against water in a similar approach with the dialysis method.

2.4. Characterization of IND-loaded nanoparticles

2.4.1. Particle size measurement and morphology

The particles size and the polydispersity index at different temperatures were determined by dynamic light scattering (DLS) measurements. The equipment consisted of a Delsa Nano Submicron Particle Size Analyzer (Beckman Coulter Inc., Brea, USA) with a 30 mW laser diode, wavelength of 658 nm and a size range of 0.6 nm − 7 μm. The auto-correlation function is automatically calculated. The measurements were performed at a scattering angle of 165° and at 25 °C and 45 °C, with an equilibration time of 300 s