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Crosslinked polysaccharide nanocapsules: Preparation and drug release properties

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Abstract

Crosslinked polysaccharide and composite polysaccharide capsules with diameters ranging from 200 nm to several microns and wall thicknesses of several tens of nanometers have been fabricated by interfacial polymerization of methacrylated *N*,*N*-diethylaminoethyl dextran (DdexMA) and DdexMA-vinyl terminated polylactide macromonomers (PLAM). In this method, chloroform droplets or PLAM-containing chloroform droplets were dispersed in water, on which water soluble DdexMA was polymerized to form closed shell structure. Their hollow nature was confirmed by confocal laser scanning microscopy and transmission electron microscopy. Dynamic light scattering revealed that these capsules possess good stability against coagulation during storage. Fourier transform infrared and elemental analysis found that the DdexMA capsules were actually composed of crosslinked DdexMA, while the DdexMA–PLAM capsules were composed of the crosslinked DdexMA–PLAM copolymers and PLAM. By dissolution of ibuprofen in the chloroform droplets, drug-loaded capsules were also fabricated. It was found that the loaded drug could be released again in a sustained manner for up to 100 h. The capsule walls had a prominent effect in slowing down the drug release rate, particularly for the DdexMA–PLAM capsules.

Keywords: Polysaccharide; Nanocapsules; Interfacial polymerization; Ibuprofen; Drug release

1. Introduction

Biopolymer nanocapsules have a wide variety of applications, particularly in drug delivery systems and in protection of biologically active materials, e.g., enzymes, drugs or peptides [1]. Compared with their solid counterparts, such as nanospheres, nanocapsules possess the advantages of relatively lower density, less consumption and higher loading capacity. Several approaches to capsule fabrication are available for drug delivery systems, including layer-by-layer self-assembly [2,3], template polymerization [4–6], emulsion polymerization [7–9], phase separation [10] and interfacial reaction [11,12]. For biologically derived polymers, interfacial reactions are generally employed to fabricate their capsules. The binding forces can be derived from chemical reaction, hydrogen-bonding or charge interaction between functional groups in their molecules such as hydroxyl, amino and/or carboxylic groups. Through chemical crosslinking or ion-crosslinking, capsules with more stable structure can be obtained [13,14]. Stable capsules of smaller size (e.g., <500 nm) and narrower size distribution are generally preferred since they can be taken up by the mononuclear phagocyte system (MPS) and be accumulated in some target organs such as the liver or spleen [15]. Therefore, efforts should be still made to develop safe and facile approaches to fabrication of nanosized capsules of natural biopolymers.

For this purpose, the mechanism of emulsion polymerization, a method that can conveniently produce particles on the nanometer scale, may be referenced in interfacial reaction [16]. In that case particles are formed by polymerization of vinyl monomers within droplets which are dispersed in a non-solvent (the so-called oil in water polymerization) [17,18]. Emulsifier is generally added to reduce

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interfacial energy between the water phase and the oil phase, thus fine droplets can be stably formed. If the monomers are not soluble in the oil phase but instead in the water phase, the polymerization will take place in the water phase and on the droplet surfaces. Thus it can be regarded as a modified interfacial polymerization, which can produce hollow polymer spheres. However, using this protocol is rarely reported for biopolymers for fabrication of biodegradable capsules, because no polymerizable vinyl groups exist in the natural polymers [19].

In the present work, the modified interfacial polymerization is adopted to prepare nanocapsules composed of *N*,*N*-diethylaminoethyl dextran (DEAE-dextran, or Ddex) and/or polylactide (PLAM). Methacrylated Ddex (DdexMA) synthesized by a conjugate reaction and/or vinyl terminated PLA (PLAM) are used as polymerizable macromonomers. Composite polysaccharide nanocapsules are prepared by interfacial polymerization of DdexMA and/or PLAM using a redox initiating system. By incorporation of ibuprofen into the capsule interiors in the polymerization process, drug-loaded capsules are obtained and the drug release properties are also assessed. With a mild and less toxic fabricating environment, these biodegradable and biocompatible nanocapsules might be expected to be favorable carriers for drug delivery systems.

2. Experimental part

2.1. Materials

N,*N*-diethylaminoethyl dextran (Ddex, $M_w \sim 500{,}000$) and water soluble carbodiimide (1-ethyl-3-dimethylaminoprophyl carbodiimide, EDAC) were obtained from Sigma. Ibuprofen was purchased from Xinhua pharma chemical Co. Ltd. (Huangyan, China). Methacrylic acid (MAA) was purified by distillation under reduced pressure. All other chemicals were of analytical grade and used as-received. Vinyl terminated PLA macromonomers (PLAM, $\overline{M}_n = 2100$) were synthesized by ring opening polymerization of lactide catalyzed by stannous chloride in the presence of allyloxylethanol (AOE) as initiators [20].

2.2. Synthesis of methacrylated Ddex (DdexMA)

DdexMA was synthesized by incubation of Ddex, MAA and EDAC in phosphate buffered saline (PBS, pH 7.4) for 24 h ($T = 25$ °C) [21]. The Ddex/MAA weight ratio was 1:3 and MAA/EDAC mole ratio was 1:2. After filtration the synthesized macromonomers were dialyzed in distilled water to remove the excess MAA and EDAC and the resultant byproducts. ^{1}H NMR spectra were recorded on a NMR spectrometer (ANAVCE DMX500) with D_2O as solvent, working at 500 MHz. Elemental analysis was performed on a Flash EA-1112 elemental analyzer.

^{1}H NMR spectra of DdexMA and Ddex are shown in Fig. 1. The characteristic resonance peaks at 3.23–3.92 ppm (H_{b-f}) and 4.91 ppm (H_a) are assigned to the protons of the methylene and other five methine groups of saccharide cyclic unit. The peaks at 1.34 ppm and 1.12 ppm (H_i, H_j) are assigned to –CH_2– and –CH_3 of the ethyl group, respectively. The ^{1}H resonance signals (H_g, H_h) of –O–CH_2–CH_2–N– were merged in that of the saccharide cyclic unit. Compared with the spectrum of Ddex, three peaks appeared at 5.54 ppm ($H_{c'}$), 5.23 ppm ($H_{b'}$) and 1.76 ppm ($H_{a'}$) in the spectrum of DdexMA, which are assigned to the vinyl and methyl groups of MAA, respectively. One can thus conclude that the MA is conjugated onto the Ddex chains. According to the N/C weight ratios of Ddex (0.068) and DdexMA (0.063) detected by elemental analysis, the MAA grafting degree was calculated as 7.7 wt.%.

2.3. Preparation of DdexMA capsules and ibuprofen-loaded capsules

DdexMA (80 mg) was dissolved in 20 ml distilled water, to which 5 ml chloroform was added to form an oil in water (O/W) emulsion under mechanical agitation (600 r/m). The polymerization was then initiated by injection of 1 ml $K_2S_2O_8$ (KPS)/$NaHSO_3$ (0.4 mg:0.4 mg) solution under nitrogen atmosphere at 40 °C. After polymerizing for 8 h, chloroform was evaporated and the capsules were collected using a membrane filtration apparatus equipped with a cellulose filter having a pore size of 50 nm, followed by sufficient washing with distilled water. For the system with a foreign emulsifier, Triton X-100 was added to the initial aqueous solution with a final concentration of 2 mg/ml.

For the system of drug loading, 16 mg ibuprofen was dissolved in chloroform in the presence of Triton X-100. The same process as described above was adopted to obtain the ibuprofen-loaded DdexMA capsules. The unloaded ibuprofen was quantified by UV measurement after the supernatant was separated by nanofiltration equipped with a cellulose membrane having a pore size of 50 nm. The drug loading efficiency was expressed as the weight ratio of ibuprofen in capsules to its total mass.

2.4. Preparation of DdexMA–PLAM capsules and ibuprofen-loaded capsules

DdexMA (40 mg) was dissolved in 20 ml distilled water which contained 2 mg/ml Triton X-100. Forty milligrams PLAM was dissolved in 5 ml chloroform, which was then poured into the aqueous solution to form an O/W emulsion under mechanical agitation (600 r/m). The polymerization was then initiated by injection of 1 ml $K_2S_2O_8$ (KPS)/$NaHSO_3$ (0.4 mg:0.4 mg) solution under nitrogen atmosphere at 40 °C. After polymerizing for 8 h, the organic solvent was evaporated. The capsules were washed with distilled water by ultrafiltration process as described above. For drug loading, 16 mg ibuprofen was added to the PLAM chloroform solution. The same process as described above was adopted to obtain the ibuprofen-loaded DdexMA–PLAM capsules.

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