Colloids and Interface Science Communications 2 (2014) 15–18

Contents lists available at ScienceDirect

Colloids and Interface Science Communications

journal homepage: www.elsevier.com/locate/colcom

Rapid Communication

Synthesis and Properties of Inulin Based Microgels

Nurettin Sahiner [a,*], Selin Sagbas [a,b], Hiroaki Yoshida [c,d], L. Andrew Lyon [c,d]

[a] Department of Chemistry, Faculty of Science & Arts, Canakkale Onsekiz Mart University, Terzioglu Campus, 17100 Canakkale, Turkey
[b] Nanoscience and Technology Research and Application Center (NANORAC), Faculty of Science & Arts, Canakkale Onsekiz Mart University, Terzioglu Campus, 17100 Canakkale, Turkey
[c] School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA
[d] Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA

ARTICLE INFO

Article history:
Received 26 June 2014
Received in revised form 1 August 2014
Accepted 4 August 2014
Available online 25 October 2014

Keywords:
Inulin
Microgels/nanogel
Biodegradable polymers
Quantum dots
Natural polymers
Polysaccharide particle
Biocompatible
Drug delivery
Fluorescent composite
Soft fluorescent composite

ABSTRACT

Cross-linked inulin (X-inulin) microparticles were synthesized in reverse micelles using water-in-oil microemulsion polymerization. Linear inulin was crosslinked with divinyl sulfone (DVS) in a sodium bis(2-ethylhexyl) sulfosuccinate (AOT) inverse microemulsion under basic conditions. These particles were demonstrated to be excellent scaffolds for the in situ synthesis of CdS quantum dots (Q-dots). The inulin-based particles were shown to be non-cytotoxic in fibroblast cell culture, and degradable under acidic and basic conditions. Furthermore, gallic acid and caffeine were used as model drugs for loading and release studies from these particles, illustrating their potential as drug carriers with controlled release.

Inulin, a β(2-1) linked polysaccharide of D-fructose, is a naturally-occurring, linear biopolymer found in many plants [1,2]. Inulin is used in various dairy products as a texture improvement agent and is found in a variety of vegetables and fruits such as onion, garlic, banana and chicory [3,4]. Inulin is assumed to be an important food additive due to prebiotic and other protective effects [1–4]. Therefore, the degradation of inulin as food additive is affected by the food processing temperature and the pH of the medium [5–7]. The advantages of inulin are safety, nontoxicity, ability to form gels, hydrophilicity, biocompatibility, and biodegradability [8–10]. Polysaccharides are often used to prepare microparticles because of their excellent properties for nutritional and medicinal applications. Natural biopolymers such as polysaccharides have been widely used as active agent carriers i.e., in drug delivery systems, tissue engineering, pharmaceuticals, and cosmetics [9,11–14]. Moreover, hydrogel microparticles with high water absorption capacity can also interact with active agents such as drugs via suitable functional groups for controlled delivery [12].

In this study, we describe the preparation and characterization of X-inulin particles and some of their applications. Cross-linked inulin hydrogel particles were synthesized using water-in-oil microemulsion system according to the previously reported method [13] that was the same as preparation of hyaluronic acid, carboxymethyl cellulose and κ-carrageenan particles [14]. Degradation of X-inulin particles was investigated by placing about 20 mg of dried X-inulin particles in a 10 mL buffer solution at pH 2.4 and 10.9 prepared from sodium citrate and hydrochloric acid, and sodium phosphate (Na_2HPO_4) and NaOH, respectively in water bath at 37 °C under constant shaking. At certain time intervals, the particles were removed from buffer solutions, centrifuged at 10 000 rpm, and washed with water and after three cycles of centrifugation in acetone, the particles were dried at 50 °C until constant weight was attained. Finally, the X-inulin weight losses against incubation time were graphed. To prepare Q-dots within X-inulin particles, 0.2 M 40 mL $CdCl_2$ solution was prepared, and to this solution, 50 mg X-inulin particles were added under constant stirring rate (400 rpm). After 12 h, Cd(II) loaded microgel particles were separated by centrifugation for 20 min at 10000 rpm, and washed with DI water several times (at least three times). Then Cd(II)-loaded particles were mixed with 40 ml 0.2 M Na_2S solution and stirred at 400 rpm for 12 h at ambient temperature. The color of particles changed from white to yellow immediately, as an indication of Q-dot (CdS) formation in situ. Then Q-dot-containing particles were centrifuged and washed several times as described above.

Drug loading and drug release were carried out using GA and caffeine CA as model drugs. A weighed amount (0.5 g) of X-inulin

* Corresponding author. Tel.: +90 286 218 0018x2041; fax: +90 286 218 0533.
E-mail address: sahiner71@gmail.com (N. Sahiner).

http://dx.doi.org/10.1016/j.colcom.2014.08.003
2215-0382/

particles was used in the drug loading experiments; X-inulin particles were placed in 50 mL 500 ppm GA solution in ethanol, or in 50 mL 300 ppm aqueous CA solution, and mixed (500 rpm) for 24 h at ambient temperature. After the drug loading period, X-inulin particles were purified by centrifugation at 10 000 rpm for 20 min and dried at 40 °C for 24 h to a constant weight. To investigate the release characteristics, separate 50 mg GA- and CA-loaded dried X-inulin particles were suspended in 1 mL of phosphate buffered saline (PBS) at pH 7.4, and transferred to a dialysis membrane (molecular weight cut off <12 000 Da, Aldrich). The release tubing was then placed into a closed beaker containing 25 ml of PBS under constant stirring at 150 rpm. The amount of drug released into the PBS buffer was evaluated by UV–Vis spectrometer (T80 + UV/Vis Spectrometer, PG Ins. Ltd) at 265 nm as a function of time as both drugs have absorption maximum at 265 nm in UV–Vis spectrum in ethanol, water and PBS solutions.

Various instrumental methods were used to characterize the X-inulin microgels. The size of X-inulin microgel was determined by dynamic light scattering (DLS) (Brookhaven Ins. and Cor. 90 plus particle size analyzer). Zeta potential measurements were conducted with Zeta-Pals Zeta Potential Analyzer BIC (Brookhaven Inst. Corp.) in 0.01 M KNO_3 solution in water. Scanning electron microscopy (SEM) images of X-inulin microgel particles were obtained using a SEM (Jeol JSM-5600) with an operating voltage at 20 kV. X-inulin particles were placed onto carbon tape-attached aluminum SEM stubs at ambient temperature after coating with gold to a few nanometers thickness under vacuum. The structural characterization was done by FT-IR (Perkin-Elmer FT-IR) spectroscopy in the spectral range between 650 and 4000 cm^{-1} using attenuated total reflectance (ATR) apparatus with 4 cm^{-1} resolution. The cytotoxicity of microgels at various concentrations (0.01, 0.1, or 1 mg/mL) was evaluated by mouse NIH 3T3 fibroblast cell culture for 24 h or 72 h. The 5×10^3 cells were seeded in 96-well plates in 200 μl of Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and incubated overnight to allow primary adhesion at 37 °C in a CO_2 incubator. Then, the solution was replaced into the culture media containing 0.01, 0.1, or 1 mg/mL of microgels and incubated for 24 h or 72 h. After washing with PBS, the living cell number was measured by a Cell Counting Kit-8 (CCK-8, DOJINDO LABORATORIES, Japan), which is an improved method of MTT assay [15]. Briefly, 10 μL of CCK-8 solution was added to the 96-well plates containing 190 μL of DMEM (10% FBS) without phenol red and the plates were incubated for 24 h or 72 h at 37 °C in a CO_2 incubator. Then, the absorbance of produced formazan at 450 nm was measured by microplate reader (Synergy H4 Hybrid Multi-Mode Microplate Reader, BioTek Instruments, Inc., USA), and the living cell number was calculated by calibration curve. UV–Visible absorbance measurements of the X-inulin-CdS microgels were also completed. The X-inulin-CdS microgel composites were stacked onto a quartz substrate to form a thin film of 3 mm thickness and their absorbance values were measured. The measured absorbance (A) spectrum is presented in S3 Fig. 3(a) with an inset graph of absorption coefficient (α (cm^{-1})), which was calculated from the equation;

$$A = \alpha.d \tag{1}$$

where, d is the sample thickness. Considering that the band gap structure is direct, the optical energy gap (E_g) of the inulin-CdS was calculated from the equation [16];

$$(\alpha h\upsilon)^2 = B\left(h\nu - E_g\right) \tag{2}$$

where, B is a constant. The graph of $(\alpha h\nu)^2$ as a function of $h\nu$ is presented in S3 Figure(b).

As illustrated in Fig. 1(a), an AOT reverse micelle can accommodate inulin in basic media and upon addition of DVS, the cross-linking of linear inulin occurs via Michael addition. To confirm this crosslinking, elemental analysis results of inulin and DVS X-inulin were compared, as DVS has sulfur in its chemical structure. The sulfur content of DVS cross-linked inulin particles was 0.715 wt.%, whereas there was no sulfur detected in virgin inulin. The sulfur from DVS confirms the crosslinking of linear inulin as illustrated in Fig. 1a. The theoretical DVS content of the X-inulin particles based on the synthesis conditions was 50 mol%, whereas the experimentally determined value was ~30 mol%, suggesting that some of the added DVS did not incorporate into the particles during particle formation. To further substantiate the crosslinking of linear inulin, FT-IR spectra of linear inulin and DVS X-inulin microgels were recorded. Characteristic peaks at 1314, 1388, and 1454 cm^{-1} corresponding to the S = O modes of linked sulfones were observed (see Supporting Information Fig. S1). The inulin microgel preparation was high yielding (90 ± 5% by mass) with a range of different microgel dimensions as shown in Fig. 1b. The X-inulin microgels have broad size distribution ranging between 1 μm and 20 μm, and their particle size was measured as 720 ± 54 nm by DLS after filtering with 1.5 μm syringe filter. The surface charge of the microgels was −46.11 mV, as obtained by zeta potential measurements.

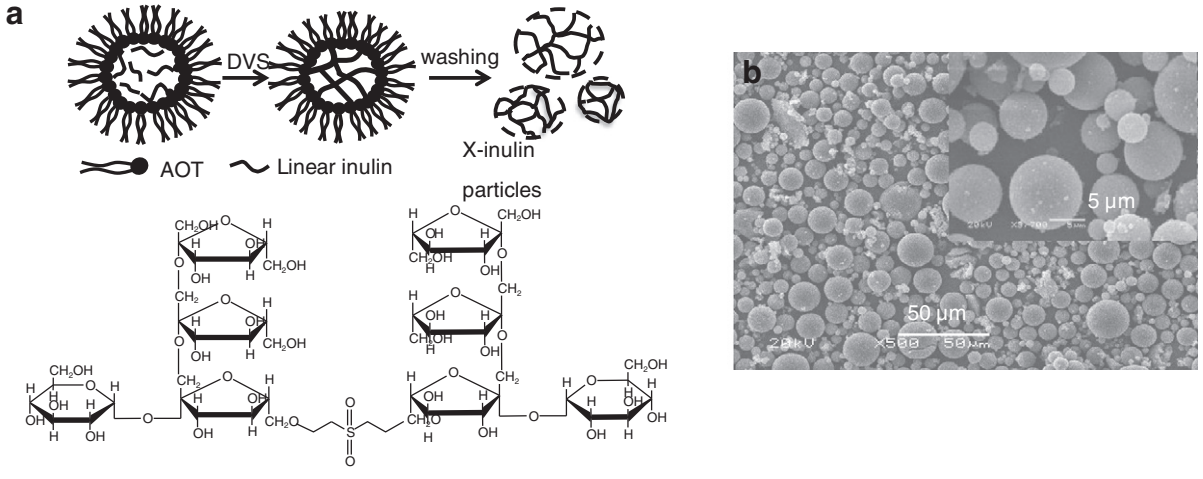

Fig. 1. Schematic illustration of (a) one pot preparation of cross-linked inulin particles in AOT reverse micelles with DVS and (b) corresponding SEM images.