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Rapid Communication

Synthesis and Properties of Inulin Based Microgels

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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.
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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

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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.

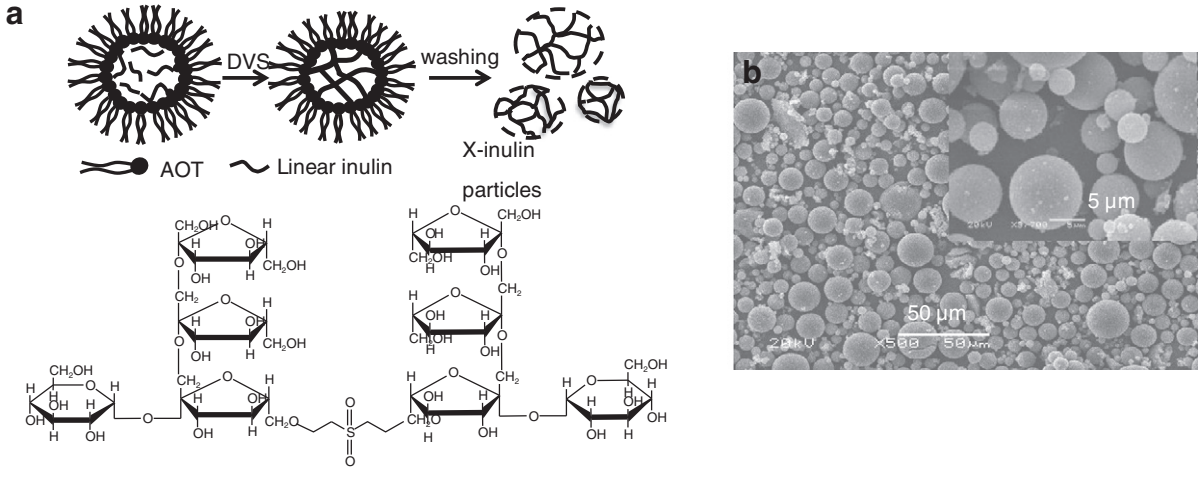

Fig. 1. Schematic illustration of (a) one pot preparation of cross-linked inulin particles in AOT reverse micelles with DVS and (b) corresponding SEM images.

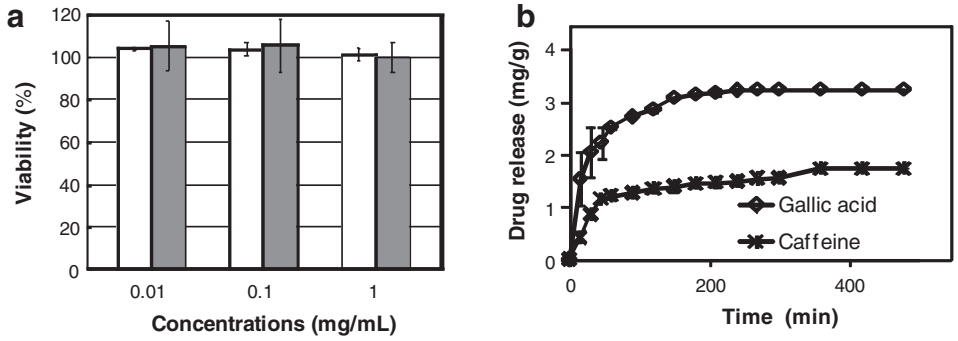

Fig. 2. (a) Cytotoxicity of X-inulin microgels [white bars 24 h and black bars 72 h incubation time]. (b) Gallic acid (GA), and caffeine (CA) release profiles from inulin microgels in phosphate buffer at pH 10.9 at 37 °C.

The cytotoxicity of X-inulin at various concentrations was evaluated in vitro by a CCK-8 assay using mouse NIH 3T3 fibroblast cells [15] as shown in Fig. 2a. After 24 h and 72 h incubation of the fibroblasts with the microgels, the metabolic activity did not change, even at high microgel concentrations (1 mg/ml) after 72 h incubation. In addition, we did not observe cell detachment from the plates or changes in cell shape under any conditions (see Supporting Information, Fig. S2). These results clearly suggest that the microgels are biocompatible in fibroblast cell culture.

Plant-derived polyphenolic compounds have many pharmaceutical properties, and GA (3,4,5-trihydroxibenzoic acid) is a natural phenolic acid obtained from a wide range of plants e.g., oak bark, sumac etc., and plant products such as tea, wine etc. [17–19]. GA has been reported to have antioxidant, anti-mutagenic, anti-viral, anti-inflammatory, anti-bacterial, and anti-carcinogenic properties [19,20]. CA is a psychostimulant and psychoactive substance found naturally in many foods and drinks. The consumption of certain amounts of CA by most people has largely positive effects such as increasing alertness, mental performance, positive mood and reducing fatigue [21,22]. To determine the potential of X-inulin microgels as drug delivery vehicles, we chose GA and CA as model drugs. Due to their low molecular weights, 170.12 and 194.19 g/mol for GA and CA respectively, they can be readily loaded into X-inulin microgels by absorption. As illustrated in Fig. 2b, both model drugs were loaded and released from X-inulin microgels. To study drug release from X-inulin microgels, the drugs were loaded for 24 h from ethanol for GA, and from water for CA which is moderately soluble in water (2 g/100 mL at room temperature). Then the loading capacities of the particles were measured with UV–Vis spectroscopy at 265 nm using a previously constructed calibration curve. It was determined that the equilibrium loading capacities were 3.4 mg GA/g and 1.75 mg CA/g. As shown in Fig. 2b, about 80% of the loaded drugs were released during the initial burst, followed by a prolonged release of remaining drugs over a few hours in PBS. These results indicate that X-inulin microgels can be used for loading and release of both acidic (GA) and basic (CA) characteristic drugs.

It is common knowledge that the degradation of inulin and fructo-oligosaccharides is greatly affected by pH and temperature [6,7]. Therefore, the degradation of X-inulin microgels was studied at 37 °C at pH 2.4 and 10.9.

As illustrated in Fig. 3a and b, in about 10 days almost 40% weight loss can occur in acidic and basic pH. These two pH values were chosen as they are very close to the pH of the stomach and the intestines. The results presented here are very important as they demonstrate control of the degradation of the x-inulin particle by the amounts of cross-linker used during microgel preparation.

To demonstrate the versatility of inulin microgels further, CdS Q-dots were prepared via absorption of Cd(II) ions from aqueous solution followed by reaction with Na_2S solution in water within X-inulin microgel matrices. It is well-known that Q-dot-containing microgels have great potential in diagnostics, bioimaging, photonics, optoelectronics, catalysis and sensors because of their tagging of biological macro/molecules, drug screening and medical diagnostic properties [23,24]. Fig. 4a depicts the in situ Q-dot preparation within inulin microgels. From TGA analysis, the CdS content of X-inulin composites was 3.36 wt.% (see Supporting Information Fig. S4).

The Cd(II)-loaded X-inulin microgels turned light yellow immediately upon reaction with S^{2-} ions as an indication of CdS Q-dot formation. It is noteworthy that CdS nanoparticles did not elute from the microgels after several centrifugation/wash cycles. Q-dot containing X-inulin microgels also displayed no observable cytotoxicity, as illustrated in Fig. 4b. Even at very high concentrations (1 mg/mL), ~100% cell viability was maintained (see Supporting Information Fig. S2). These results further confirm that X-inulin microgels are versatile and have great potential in biomedical applications. The optical characterization of CdS within X-inulin microgels was carried out using Eqs. (1) and (2), and E_g values were determined by

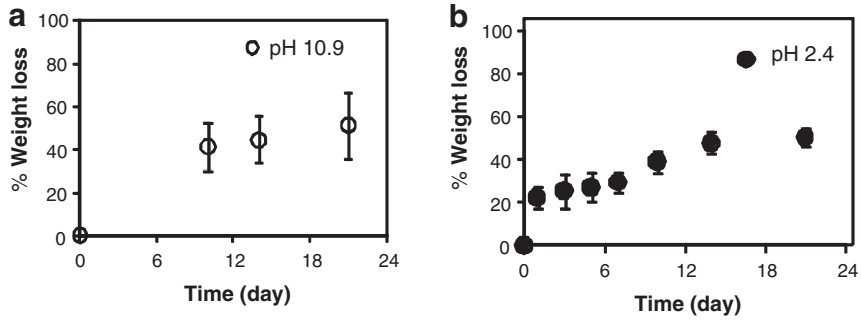

Fig. 3. (a) Degradation of X-inulin microgels in citrate buffer at pH 2.4 and (b) in phosphate buffer at pH 10.9 at 37 °C.

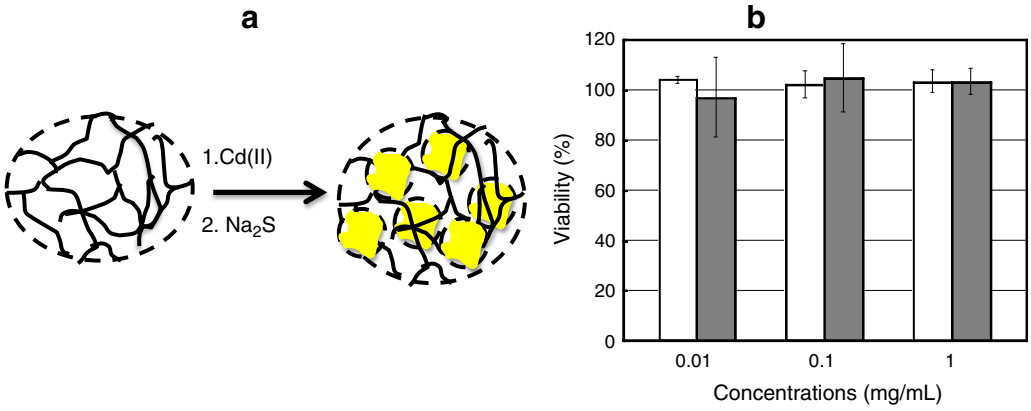

Fig. 4. (a) The representation of in situ CdS quantum dot preparation within X-inulin microgels and (b) Cytotoxicity test of X-inulin microgels [white bars 24 h and black bars 72 h incubation time].

fitting a linear function to $(\alpha h\nu)^2$ and extrapolating it to $(\alpha h\nu)^2$ at zero value (see Supporting Information Fig. S3). E_{g1} can be attributed to the energy band gap of X-inulin, whereas E_{g2} can be interpreted as the energy band gap of CdS, which is compatible with the values reported for CdS films in the literature, between 2.1 and 2.9 eV [25,26].

To sum up, here we demonstrated a facile one-pot synthesis of X-inulin microgels from linear inulin employing AOT water-in-oil microemulsion system with high yield (over 90%). Although the size distribution of microgels is broad, the desired size selection for different biomedical applications could be accomplished via simple filtration or centrifugation. We further demonstrated that X-inulin microgels are not toxic, and can be used as drug delivery devices by simple absorption e.g., of acidic (GA) and basic (CA) drugs, and then released into aqueous solution. Additionally, X-inulin microgels can even be used for in situ Q-dot preparation for potential cell targeting and imaging applications.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.colcom.2014.08.003.

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