



Preparation of active polysaccharide-loaded maltodextrin nanoparticles and their stability as a function of ionic strength and pH

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ABSTRACT

Active polysaccharides (APs) are known to have a wide variety of biological functions, such as anti-viral, anti-oxidant, hypoglycemic, and lipid-lowering effects. However, the extremely low bioavailability of APs restricts their bioactivity in vivo; therefore, the development of a biopolymer nanoparticle carrier is a promising solution. For the first time, we have successfully prepared maltodextrin nanoparticles (MNPs) with controllable particle sizes. The influence of different emulsifier types and volume ratios of a maltodextrin solution to absolute ethanol on the morphology and size of the MNPs was examined, and the smallest MNPs (30–90 nm) were obtained when the volume ratio was 1:10 and the emulsifier was Tween 80. Furthermore, tea, pumpkin, and balsam pear polysaccharides were loaded on the MNPs with loading efficiencies of 63.5, 68.7, and 72.1%, respectively. Compared with the MNPs, the AP-loaded MNPs were more stable in a high salt content, or under a gastric pH of 1.2 and physiological pH of 7.4 conditions. These MNPs may serve as effective nanocarriers by delivering APs to enhance their bioavailability.

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1. Introduction

Nanoparticles have attracted much attention due to their unique properties, when compared to bulk materials, including a sub-micron size and large surface-to-volume ratio. There is growing interest in using natural biopolymers, like starch, cellulose, chitosan, protein, and dextrin, as precursor materials in the synthesis of nanoparticles for various biomedical and industry applications, such as drug delivery carriers (Kaneo, Taguchi, Tanaka, & Yamamoto, 2014; Rodrigues & Emeje, 2012), contrast agents (Gonçalves et al., 2013), and biodegradable edible film (González & Alvarez Igarzabal, 2015; Shi, Wang, & Adhikari, 2013). Maltodextrin, a saccharide-based polymer containing D-glucose units linked by α -1, 4 or α -1, 6 glycosidic bonds, is produced by the partial hydrolysis of starch. Due to its good biocompatibility and degradability attributes, maltodextrin has been widely used in the biomedicine and food industries (Carvalho, Gonçalves, Gil, & Gama, 2007).

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Currently, several researchers have explored a variety of synthetic methods to prepare polymer nanoparticles, such as acid or enzymatic hydrolysis, emulsion cross-linking, microemulsion, and self-assembly processes (Le Corre & Angellier-Coussy, 2014; Manchun, Dass, & Sriamornsak, 2014; Motornov, Roiter, Tokarev, & Minko, 2010). Manchun et al. (2014) reported synthesized dextrin nanoparticles, 120–200 nm in size, by an emulsion cross-linking technique using glyoxal as a cross-linker. They suggested that the surfactants had a significant effect on the size of the particles. However, the emulsion cross-linking technique usually requires a large amount of mechanical energy generated by an ultrasound generator or high-pressure homogenizer, possibly producing pollution (Burapapadth, Takeuchi, & Sriamornsak, 2012). Gonçalves et al. (2010) reported dextrin nanoparticles with particle sizes of around 20–200 nm that were synthesized by self-assembly in water, through an association with hexadecanethiol. However, this method did not allow tuning control of the particle sizes.

Anti-solvent precipitation is a promising method because it is simple, has a short cycle, and produces a high yield with no pollution. Moreover, it has been widely used in industry to produce nanoparticles for pharmaceutical purposes (Joye & McClements, 2013). However, there are no reports on the preparation of maltodextrin nanoparticles (MNPs) through anti-solvent nanoprecipitation.

Active polysaccharides (APs) are one kind of active component, and widely present in various plants, such as tea, pumpkin, and balsam pear. A large number of experiments have shown that polysaccharides have a wide variety of biological functions, including immune regulation, anti-viral, anti-oxidant, and anti-tumor effects, and hypoglycemic and lipid-lowering effects (Gui, Fu, Hou, & Jin, 2007). However, the extremely low bioavailability of APs restricts their bioactivity in vivo. A nanoparticle delivery system is a system in which nanocarriers are used to encapsulate or adsorb bioactive compounds to either enhance their absorption in the gastrointestinal tract by active endocytosis, or improve bioactivity in the body circulation by specific targeting (Phan et al., 2015; des Rieux, Fievez, Garinot, Schneider, & Preat, 2006). Therefore, the bioactive compounds loaded in nanoparticles could increase

The yield was calculated by dividing the weight of the freeze-dried precipitate by the initial dry weight of the maltodextrin.

To prepare the AP-loaded MNPs, three polysaccharides (TP, PP, and BP) (0.5%, w/w) were dissolved in the maltodextrin solution. Tween 80 was selected as the emulsifier, and the volume ratio of the maltodextrin/AP mixed solution and absolute ethanol was 1:10. Samples of the AP-loaded nanoparticles were obtained by centrifugation, and rinsed with absolute ethanol three times. All of the supernatants were mixed together and quantified spectrophotometrically. Using a standard curve that was plotted using different concentrations of the AP, the concentration of the unknown AP was estimated. The TP, PP, and BP-loaded MNPs were named MNPs-TP, MNPs-PP, and MNPs-BP, respectively, while the loading efficiency was calculated by the following equation [Eq. (1)]:

$$\text{Loading efficiency (\%)} = (\text{Total content of AP} \times 100 - \text{Content of AP in supernatant}) / \text{Total content of AP} \times 100 \quad (1)$$

bioavailability and bioactivity (Roger, Lagarce, Garcion, & Benoit, 2010). Research on biopolymer nanocarriers has become a hot topic recently. Furthermore, the stability of the nanocarriers is also important to allow successful delivery to the target site (Tang et al., 2014).

The aim of this study was to develop MNPs with controllable particle sizes, using anti-solvent nanoprecipitation to encapsulate active polysaccharides in order to enhance their bioavailability. In our preliminary examinations, we found that an emulsifier was necessary in order to maintain the shape and size of the particles. Therefore, in this study, three different kinds of emulsifiers (SDS, Span 80, and Tween 80) were used to prepare the MNPs. The morphological characteristics, size distribution, and structure of the MNPs prepared with the different emulsifiers in different volume ratios of the aqueous maltodextrin solution to the absolute ethanol (anti-solvent) were investigated. Furthermore, the stability of the AP-loaded MNPs in a high salt content, or under a gastric pH of 1.2 and physiological pH of 7.4 conditions were also investigated.

2. Materials and methods

2.1. Materials

The maltodextrin (dextrose equivalent, DE 10), sodium dodecyl sulfate (SDS), Tween[®] 80, and Span[®] 80 were obtained from Sigma Chemical Company (St Louis, Missouri, USA). The absolute ethanol was of analytical grade and was used without further purification. The tea polysaccharide (TP), pumpkin polysaccharide (PP), and balsam pear polysaccharide (BP) were provided by the Nanjing Spring and Autumn Biological Engineering Co., Ltd. (Nanjing, China).

2.2. Sample preparation

To prepare the MNPs, maltodextrin was dissolved in the water phase to obtain a final concentration of 5% (w/w), and then a 0.5% (w/w) emulsifier (SDS, Tween 80, and Span 80, respectively) was added to the suspension. A fixed quantity of absolute ethanol (80, 100, and 120 mL) was added drop-wise to 10 mL of the above suspensions, and continually stirred using a magnetic stirrer for 1 h at a constant rate of 600 rpm. Samples of the nanoparticles were obtained by centrifugation, rinsed with absolute ethanol three times to remove excess water and emulsifier, and then freeze dried.

2.3. Transmission Electron Microscopy (TEM)

Transmission electron micrographs of the MNPs were taken using a Hitachi 7650 TEM (Hitachi, Tokyo, Japan) with an acceleration voltage of 80 kV. A drop of the MNPs dispersed in double-distilled water was placed on a copper grid and lyophilized.

2.4. Dynamic Light Scattering (DLS)

The average size, size distribution, and zeta potential of the samples were estimated by dynamic light scattering (DLS) using a Malvern Zetasizer Nano (Malvern Instruments Ltd., Worcestershire, UK) equipped with an He–Ne laser (0.4 mW; 633 nm) and a temperature-controlled cell holder. The measurements were performed following the method of Pignatello et al. (2006), and the mean intensity-weighted diameter and zeta potential were recorded.

2.5. Molecular weight distributions

The molecular weight distributions of the MNPs were analyzed using high-performance size-exclusion chromatography (HPSEC) (Shimadzu LC-20A, Kyoto, Japan) equipped with a refractive index (RI) detector. The HPSEC was carried out according to Jiang, Campbell, Blanco, and Jane (2010) with some modifications. The MNPs were dissolved in ultrapure water and mechanical stirring for 10 min, and an aliquot (50 µL) of the dispersion was filtered through a nylon membrane (0.25 µm pore size). The HPSEC-RI system consisted of a pump (LC-20AT), an injection valve (SIL-20A), and an RI detector. PL-aquagel-OH 30 analytical columns (Agilent Technologies, California, USA) with a guard column were used to analyze the molecular weights of the samples, and the temperature of the columns (PL-aquagel-OH 30) was maintained at 35 °C. Distilled-deionized water filtered through a membrane with a pore size of 0.25 µm was used as the eluent, with a 1 mL/min flow rate. Pullulan standards (Mw 342, 1320, 6200, 10600, and 21700) were used as references to determine the molecular weights of the MNPs.

2.6. Fourier Transform Infrared Spectroscopy Analysis (FTIR)

The FTIR spectra were recorded using a Nicolet 6700 spectrometer (Thermo Fisher Scientific Inc., Massachusetts, USA), while the SNP samples were collected using the KBr pellet method. The

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