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# A comparative study of size-controlled worm-like amylopectin nanoparticles and spherical amylose nanoparticles: Their characteristics and the adsorption properties of polyphenols

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ABSTRACT

Polyphenols are known to have potent antioxidant capacity and other health-beneficial bioactivities. However, extremely low absorption rate of polyphenols restricts their bioactivity in vivo. Development of biopolymer nanoparticle carrier is a promising solution. For the first time, we have successfully prepared worm-like amylopectin nanoparticles (APNPs) and spherical amylose nanoparticles (AMNPs) using fractionated amylose and amylopectin from potato starch. Additionally, adsorption kinetics and adsorption isotherms of three polyphenols (procyanidins, epicatechins and catechins) on AMNPs and APNPs were investigated. We found that procyanidins, epicatechins, and catechins could bind to AMNPs at levels of up to 1.2, 1.5, and 1.4 g/g, respectively, while the APNPs demonstrated higher adsorption amounts of 1.4, 4.3, and 2.2 g/g, respectively. Furthermore, the particle size of polyphenol-loaded nanoparticles was not significantly changed. The results suggested that APNPs and AMNPs can be applied as an effective nanocarrier by delivering active compounds for nutraceutical and pharmaceutical industries.



## 1. Introduction

Due to their submicron size, high surface-to-volume ratio, and fine biological compatibility, starch-based nanoparticles have been widely used for various biomedical and industry applications, such as nanoparticle-based delivery systems (Rodrigues & Emeje, 2012; Simi & Abraham, 2007) and biodegradable edible films (Shi, Wang, Li, & Adhikari, 2013). In general, the methods used to prepare nanoparticles can be categorized as top-down (such as homogenization and milling) and bottom-up methods (such as self-assembly or nanoprecipitation). Compared with top-down methods, the bottom-up methods are more attractive, as there is no need for specialized equipment, the associated costs are reasonably low, and the risk of sample contamination is often significantly reduced.

At present, most starch-based nanoparticles by bottom-up methods are prepared using native starch as the precursor material. Few studies have reported the preparation of starch nanoparticles using amylose or amylopectin. Among the existing literature, Ghaeb, Tavanaia, and Kadivar (2015) reported that amylose and amylopectin nanoparticles with particle sizes of around 100–300 nm were synthesized by electrospraying. Similarly, Dong, Chang, Wang, Tong, and Zhou (2015) synthesized starch nanoparticles ranging from 160 to 300 nm by precipitation from amylose. However, methods described in the literature still demonstrate some limitations, such as significant energy consumption and relatively large particle sizes; moreover, natural starch usually consists of 25% amylose (linear starch polymers) and 75% amylopectin (branched starch polymers) (Xue, Subramanyam, Shi, Campbell, & Hartzer, 2010). The preparation of starch nanoparticles using fractionated amylopectin and amylose by nanoprecipitation has not been reported. Interestingly, in our preliminary experiments, the nanoparticles fabricated by amylopectin fractionated from potato starch exhibited a worm-like structure. This is atypical; to our knowledge, the morphology of starch nanoparticles prepared by various methods are almost always spherical or oval in shape, and there has been no similar reporting of worm-like starch nanoparticles up to now. Moreover, there is no research on the binding properties of the worm-like starch nanoparticles.

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 to improve bioactivity in body circulation by specific targeting (Phan et al., 2015). Therefore, the bioactive compounds or drugs encapsulated in nanoparticles could increase

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bioavailability and bioactivities (Roger, Lagarce, Garcion, & Benoit, 2010). Polyphenols are predominantly plant secondary metabolites, which exist in fruits and vegetables widely. As "lifespan essential" compounds, polyphenols exhibit potential benefits to human health in preventing the development of certain diseases, such as cardiovascular diseases and cancers, and maintaining human well-being (Saura-Calixto, Serrano, & Goñi, 2007). Procyanidins are oligomers and polymers of flavan-3-ols (Prior & Gu, 2005). Epicatechins and catechins are the primary bioavailable forms of the procyanidins. They are known to have potent antioxidant capacities and may reduce the risk of chronic diseases, such as cardiovascular diseases and cancers (Santos-Buelga & Scalbert, 2000). However, they have low absorption and permeation rates due to passive diffusion, leading to extremely low oral bioavailability (Déprez et al., 2000; Gu, House, Rooney, & Prior, 2007).

In order to resolve this issue, several researchers have explored the binding of polyphenols to nanoparticles. For instance, Zou, Li, Percival, Bonard, and Gu (2012) reported that cranberry procyanidins were encapsulated in the zein protein to form nanoparticles, and the resultant procyanidins-zein nanoparticles increased procyanidin solubility in aqueous system. Phan et al. (2015) demonstrated that water-soluble polyphenols had a high-binding affinity to cellulose. Additionally, poly lactic co-glycolic acid nanoparticles, chitosan nanoparticles, and protein nanoparticles were all shown to encapsulate curcumin in order to enhance its absorption and bioavailability (Anand et al., 2010; Teng, Luo, & Wang, 2012). However, there is no research on the polyphenol binding properties of starch nanoparticles. Therefore, the aim of this study was to prepare AMNPs and APNPs that were fractionated from potato starch and to determine their different morphological characteristics, size distribution, zeta potential, crystalline structure, and thermal characteristics. Furthermore, the binding rate and capacity of polyphenols (procyanidins, epicatechins, catechins) to AMNPs and APNPs were also investigated.

## 2. Materials and methods

### 2.1. Materials

Potato starch (PS, 28.0% amylose content) was supplied by Tianjin Dingfung Starch Development Co., Ltd. (Tianjin, China). Analytical grade absolute ethanol was used without further purification. Procyanidins (purities >98%), epicatechin (purities >98%), and catechins (purities >98%) were provided by Nanjing Spring and Autumn Biological Engineering Co., Ltd (Nanjing, China).

### 2.2. Preparation of starch nanoparticles

The amylose and amylopectin of potato starch were isolated according to the method developed by Li et al. (2014) with some modifications. Potato starch (10 g) was slowly added to distilled water (100 mL), and was stirred vigorously. The suspension was heated at 65 °C in a water bath for 30 min, and then the supernatant containing amylose and the precipitation of amylopectin was isolated by paper pulp filtration. The butanol (50 mL) was added to the supernatant containing amylose. After 2 h, this mixture was centrifuged at 3000*g* for 10 min and washed with absolute ethanol to remove the butanol, leading to the precipitation of pure amylose. To obtain the amylopectin, methanol (100 mL) was added to the precipitation of amylopectin and mixed, prior to being centrifuged at 3000*g* for 10 min, and then washed with absolute ethanol to remove the methanol. The separated amylose and amylopectin fraction was then dried at 40 °C for 2 h.

Following this, the amylose and amylopectin (1%, w/v), now contained in 90% dimethyl sulfoxide, was heated in a boiling water bath and stirred constantly for 1 h. After dissolution, 4 volumes of ethanol were added and the mixture was then centrifuged at 6000*g* for 15 min. The supernatants were discarded and the sediment was washed with absolute ethanol, followed by re-centrifugation. Then, the sediment was dried at 40 °C for 2 h. The amylose and amylopectin solutions (1%, w/v) were reheated in a boiling water bath and stirred constantly for 30 min to complete gelatinization. Afterwards, a fixed quantity of absolute ethanol (30, 40, 50 mL) was added drop-wise into 10 mL of gelatinized amylose and amylopectin solutions, which was stirred continually with a magnetic stirrer for 2 h at a constant rate of 600 rpm. In the final phase of preparation, the amylose nanoparticles (AMNPs) and amylopectin nanoparticles (APNPs) were obtained by centrifugation, rinsed with absolute ethanol three times to remove excess water, and then freeze dried. AMNPs 1:3, 1:4 and 1:5 represent AMNPs prepared by addition of 3, 4, and 5 volumes of absolute ethanol to amylose solution, respectively. APNPs 1:3, 1:4 and 1:5 represent APNPs prepared by addition of 3, 4, and 5 volumes of absolute ethanol to amylopectin solution, respectively.

The amylose contents of the fractionated amylose and amylopectin both before and after precipitation were determined according to the iodine staining as described by Miao, Zhang, and Jiang (2009). The amylose content was calculated from a standard curve prepared using mixtures of pure potato amylose and amylopectin (over the range 0–100% amylose). The amylose content of the fractionated amylose and amylopectin before precipitation was 95.2% and 5.6%, respectively. After nanoprecipitation, the amylose content of the AMNPs 1:3, 1:4, and 1:5 was 96.2%, 96.7%, and 96.5%, respectively. And the amylose content of the APNPs 1:3, 1:4, and 1:5 was 5.1%, 5.4%, and 5.2%, respectively.

### 2.3. Transmission electron microscopy (TEM)

Transmission electron micrographs of the nanoparticles were taken with a Hitachi 7650 TEM with an acceleration voltage of 80 kV. The nanoparticles were deposited on a carbon-coated grid without any treatment.

### 2.4. Dynamic light scattering (DLS)

The average size, polydispersity index (PDI) and size distribution of the nanoparticles were estimated by dynamic light scattering (DLS) using a Malvern Zetasizer Nano (Malvern Instruments Ltd., UK) equipped with a He–Ne laser (0.4 mW; 633 nm) and a temperature-controlled cell holder. The intensity of the scattered light was detected at 90° to the incident beam. The measurements were performed in samples diluted in deionized water and analyzed at 25 °C (Pignatello et al., 2006). The mean intensity weighted diameter was recorded as the average of three mean measurements.

### 2.5. Determination of zeta potential

The nanoparticle suspensions (0.01%, w/v; pH 7.0) were measured for their electrophoretic mobility by laser Doppler velocimetry using a Malvern Zetasizer Nano (Malvern Instruments Ltd., UK), following the method reported by Teng et al. (2012) and using compatible fold capillary cuvettes provided by Malvern Inc. (Malvern, UK). The electrophoretic mobility of each sample was measured three times, and at least 12 runs were performed in each measurement.

### 2.6. X-ray diffraction

The crystalline structures of samples were analyzed using an X-ray diffractometer (D8-ADVANCE, Bruker AXS Model, Germany)