Development of a nanoparticle delivery system based on zein/polysaccharide complexes

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Abstract: Zein, an alcohol-soluble protein in corn, can self-assemble into spherical nanoparticles, which makes it an ideal carrier material for the encapsulation of hydrophobic bioactive molecules. However, zein nanoparticles easily aggregate in water and are sensitive to enzymatic degradation in the digestive system. A strategy to overcome their limitations is the incorporation of polysaccharides as a second polymer layer that provides stability to zein nanoparticles. This review introduces the characteristics of zein as they relate to understanding the formation of zein/polysaccharide nanoparticles. Particular attention is paid to the preparation methods for the zein/polysaccharide nanoparticles, as well as to the morphological observation methods and detection mechanism. Moreover, the properties and applications of zein/polysaccharide nanoparticles are highlighted.

Keywords: application, formation, nanoparticles, polysaccharides, zein

1 INTRODUCTION

There is an increasing demand for high-quality and flavorful food that is also nutritious, functional, and safe. However, many nutrients and bioactivities, such as essential oils, α-tocopherol, resveratrol, and curcumin, are not soluble in water and are sensitive to pH or light or heat, resulting in low bioavailability and, thus, limited nutritional value and bioactivity. Attempts have been made to produce nanodelivery systems to encapsulate those substances to extend their utilization (Chen, Remondetto, & Subirade, 2006; He et al., 2011; Kasaai, 2018; Livney, 2010; Mcclements, Decker, & Weiss, 2007). The materials we choose to fabricate the nanodeliveries should be natural, biodegradable, and biocompatible to meet the demand for food safety.

Zein, a protein from maize, has proven to be a good material for the fabrication of delivery systems such as nanoparticles (Cheng, Ferruzzi, & Jones, 2019; Gagliardi et al., 2019; Gonçalves Da Rosa et al., 2020; Merino et al., 2019; Zhang, Khan, Cheng, & Liang, 2019), films/coatings (Boyacı et al., 2019; Kashiri, López-Carballo, Hernández-Muñoz, & Gavara, 2019; Moreno, Orqueda, Gómez-Mascaraque, Isla, & López-Rubio, 2019; Spasojević et al., 2019; Zhang et al., 2015; Zink, Wyrobnik, Prinz, & Schmid, 2016), and emulsions (Boostani et al., 2019; Chuacharoen & Sabliov, 2019; Zou, Thijssen, Yang, & Scholten, 2019). Nutrients, bioactives, and drugs, especially hydrophobic substances, could be encapsulated into delivery systems based on zein to improve their stability, bioactivity, and bioavailability.

However, zein as a sole material is generally not suitable for technological applications because it is poorly water soluble and cannot efficiently control the release of encapsulated compounds. Polysaccharides as natural materials have been used widely in the food industry, which are highly stable, biocompatible, and biodegradable. Because of their native charges (ionic or nonionic) and various groups (hydroxyl, carboxyl, amino groups, etc.) on their molecular chains, polysaccharides can participate in the preparation of nanocarriers together with proteins according to the following mechanisms: chemical cross-linking, physical cross-linking, polyion complex formation, and self-assembly (Nitta & Numata, 2013). Combining zein with polysaccharides to form nanodelivery systems can provide more prospects for the utilization of hydrophobic bioactivities compared to individual polymers (Sun, Dai, & Gao, 2016). Polysaccharides such as chitosan, alginate, and pectin, among others, have already employed with zein to produce nanodelivery systems.

Although studies on zein or zein-based delivery systems such as films, fibers, gel-based system, emulsions, and micro-/nanoparticles have been reviewed by previous researchers, there has been insufficient systematic elaboration regarding zein/polysaccharide nanoparticles (Berardi, Bisharat, Alkhatib, & Cespi, 2018; Corradini et al., 2014; Kasaai, 2018; Luo & Wang, 2014; Paliwal & Palakurthi, 2014; Patel & Velikov, 2014; Tran, Duan, Lee, & Tran, 2019a, 2019b). In this work, the characteristics of zein will be discussed, and the preparation, morphology, formation mechanism, and applications of zein/polysaccharide nanoparticles will be specifically reviewed. Moreover, the possible prospects for research on zein/polysaccharide nanoparticles will be proposed.

2 CHARACTERISTICS OF ZEIN

Zein, a prolamine, is the main protein in corn and is usually obtained from corn by-products such as distiller's dried grains or corn gluten meal (Anderson & Lamsal, 2011b; Xu, Reddy, & Yang, 2007). Zein is considered as generally recognized as safe (GRAS) by the US Food and Drug Administration (Liu, Cao, Ren, Wang, & Zhang, 2019). Its special properties have captured the attention of researchers in the field of nanotechnology for the food industry (Kasaai, 2018; Luo & Wang, 2014).

2.1 Composition

Zein, which accounts for approximately 50% of the total protein content of whole grain of corn, is a mixture of many similar proteins that are categorized by solubility and molecular weights into four fractions: α-Zein, β-Zein, γ-Zein, and δ-Zein. α-Zein is soluble in 50 to 95% (v/v) 2-propanol, has a MW of 21 to 25 kDa,

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and constitutes 75 to 85% of the total zein. β-Zein is soluble in 30 to 85% 2-propanol, has a MW of 17~18 kDa, and accounts for 1 to 5% of the total zein. γ-Zein is soluble in 0 to 80% 2-propanol, is made up of 27 kDa and 18 kDa polypeptides, and accounts for 10 to 20% of the total zein. δ-Zein has the same solubility as α-Zein, but it has a MW of 10 kDa and accounts for the 1 to 5% of the total zein (Anderson & Lamsal, 2011a).

2.2 Properties

Zein is insoluble in water but is soluble in aqueous alcohol, high concentrations of urea, alkaline pH (>11), or anionic surfactants. Its solubility is ascribed to its composition consisting of more than 50% hydrophobic amino acids (Patel & Velikov, 2014). Based on small-angle X-ray scattering measurements, Matsushima et al. proposed a zein structure consisting of 9 to 10 helical segments in an anti-parallel fashion linked by glutamine-rich turns. In this model, the top and bottom loops containing glutamine were hydrophilic, while surfaces containing the a-helix sides were hydrophobic. This model explained the amphiphilic and self-assembling nature of zein. Due to its particular physicochemical properties, zein can be converted into film, fiber, and micro-/nanostructures for its utilization in the food and the pharmaceutical industry (Berardi et al., 2018; Corradini et al., 2014; Luecha, Hsiao, Brodsky, Liu, & Kokini, 2011; Paliwal & Palakurthi, 2014; Patel & Velikov, 2014; Tran et al., 2019a, 2019b).

2.3 Formation of the nanocarriers based on zein

The methods of forming nanocarriers from zein include antisolvent (Cheng et al., 2019; Zhang et al., 2019), electrospinning (Deng, Li, Feng, Wu, & Zhang, 2019; Heydari-Majd, Rezaeinia, Shadan, Ghorani, & Tucker, 2019; Wang, Zhao, Barker, Belton, & Craig, 2019), electrospraying (Miguel et al., 2019), supercritical antisolvent (Franco, Reverchon, & De Marco, 2019; Palazzo, Campardelli, Scognamiglio, & Reverchon, 2019), heat-induced self-assembly (Wang & Zhang, 2019), and evaporation induced self-assembly (Wang, Su, Schulmerich, & Padua, 2013). Although these methods have different processes, they follow a common mechanism. First, zein is dissolved in the aqueous ethanol (70 to 90%), and the zein solution is then either poured into water or hot water or is heated directly to evaporate the ethanol. During this processing, the polarity of the zein solution changes from hydrophobic to hydrophilic causing zein molecules to aggregate into nanoparticles by hydrophobic interaction. The droplet size is affected by the shear rate, ethanol concentration, and zein concentration (Zhong & Jin, 2009). Moreover, the nanostructure of zein self-assembly can be controlled by adsorbing zein molecules on hydrophobic or hydrophilic surfaces (Dong, Padua, & Wang, 2013; Padua & Wang, 2009). A possible mechanism of zein self-assembly could occur as follows: during the antisolvent process, the α-helices in the zein molecule transform into β-sheets; then, the β-sheets begin packing into nanoparticles (Wang & Padua, 2012).

Although electrospraying, electrospinning, and supercritical antisolvent co-precipitation have been used to prepare zein nanoparticles, the requirement of special instruments severely limited their applications (Franco et al., 2019; Miguel et al., 2019; Palazzo et al., 2019; Wang et al., 2019). However, electrospraying and electrospinning are better for forming nanofibers, and evaporation-induced self-assembly is more suitable for the formation of zein films.

2.4 Applications of the nanodelivery systems obtained from zein

The zein nanodelivery systems have shown their advantages in the utilization of drugs, nutrients and bioactivities, such as abamectin, ciprofloxacin, curcumin, vitamin D, fish oil, DNA, enzymes, and polyphenols, improving stability to environment changes and the gastrointestinal tract, increasing bioavailability, remaining noncytotoxic to intestinal cells, and maintaining or improving the activities. The preparation methods, particle size, and PDI have significant effects on the application of zein nanoparticles. The potential applications of zein-based delivery systems were specifically reviewed by published articles (Berardi et al., 2018; Kasaai, 2018; Paliwal & Palakurthi, 2014; Patel & Velikov, 2014; Tran et al., 2019a, 2019b). However, zein nanoparticles have poor aggregation stability when exposed to the harsh conditions, such as the isoelectric point (p*I*) of zein, high ionic strength, and high temperature. In addition, they can be quickly hydrolyzed in the stomach by pepsin, thereby releasing any encapsulated ingredients into gastric fluids, which may cause the degradation of pH- or enzyme-sensitive ingredients such as anthocyanins. The formation of zein/polysaccharide nanoparticles may help to improve the physical stability to environmental stresses and to resist digestion within the human gastrointestinal tract (Hu & Mcclements, 2015).

3 ZEIN/POLYSACCHARIDE NANOPARTICLES

3.1 Procedures of the preparation of zein/polysaccharide nanoparticles

Although zein can interact with polysaccharides by covalent or noncovalent binding, noncovalent binding is the most common. This preference occurs because (i) the Maillard reaction between proteins and polysaccharides is too complicated to control; and (ii) if cross-linking agents are involved, the processing required to remove the agents for the sake of food safety becomes more complex (Anal, Shrestha, & Sadiq, 2019; Dinh, Tran, Duan, Lee, & Tran, 2017; Xu, Melton, Williams, & Mcgillivray, 2017). So the most popular way to prepare zein/polysaccharide nanoparticles is the antisolvent method (liquid–liquid dispersion). Moreover, according to the specific processing of the antisolvent method, this method is further classified into different procedures (as shown in Figure 1 and Table 1): antisolvent precipitation, pH- or heat-inducted antisolvent precipitation, and antisolvent coprecipitation.

Antisolvent precipitation Figure. (1A): First, zein is dissolved into aqueous ethanol (70 to 90%), after which the zein solution is poured into water and zein nanoparticles are formed; second, the zein nanoparticles solution is mixed with the polysaccharides solution to form nanoparticles by electrostatic adsorption interactions between zein molecules and polysaccharide molecules. The nanostructure of zein/polysaccharide complexes is also called a core–shell structure. The core is zein nanoparticles, and the shell is polysaccharides outer layer.

pH-induced antisolvent precipitation (Figure 1A): During this processing, the pH is adjusted (generally pH 4) in both solutions before the zein nanoparticle solution and the polysaccharides solution are mixed. The p*I* of zein is approximately 6.2, and the p*I* of zein nanoparticles is approximately 5 to 6 (Hu & Mcclements, 2015; Huang et al., 2016). Thus, at pH 4, zein nanoparticles have positive charges, and the polysaccharides have negative charges. The pH-induced method provides a stronger electrostatic adsorption force between zein and polysaccharides.

Heat-induced antisolvent precipitation (Figure 1A): This performance is based on pH-induced antisolvent precipitation. After

Table 1–Summary of the studies on the delivery system based on zein–polysaccharides nanocomplexes.

Polysaccharides	Preparation	Microstructure or Morphology	Encapsulated active ingredient	Effect of delivery systems on the properties of ingredients
PGA (Sun et al., 2016; Wei, Yu et al., 2019; Sun, Chen, Dai, & Gao, 2017; Sun, Dai, & Gao, 2017a, 2017b; Sun, Yang, Dai, Chen, & Gao, 2017; Dai et al., 2018)	Antisolvent co-precipitation	Spherical or Fruit tree-like	Resveratrol Quercetagetin β-Carotene	(1) Keep ingredients amorphous in nanoparticles rather than crystalline. (2) Sustained-release in GIT. (3) Physicochemical stability was improved. (4) The release of ingredients and the droplet size were also affected by the concentration of Ca.
Alginate (Hu & Mcclements, 2015; Lee et al., 2016; Bautista et al., 2019)	Antisolvent precipitation Or pH-induced antisolvent precipitation	Spherical core–shell	Limonoids SOD	(1) The release of ingredients was controlled by pH. It is released less in SGF and more in SIF. (2) Not only provided protection for ingredients but improved their activities as well.
Chitosan (Luo et al., 2011; Li, Chen et al., 2018)	Antisolvent precipitation	Spherical core–shell	α-Tocopherol Curcumin	(1) Sustained-release in GIT. (2) Gave a better protection against gastrointestinal to ingredients. (3) Improved activities of ingredients.
Alginate/chitosan (Khan et al., 2019)	Antisolvent precipitation	Spherical core–shell	Resveratrol	(1) Resveratrol remained amorphous. (2) Improve the photostability and bioaccessibility of resveratrol. (3) Sustained-release in GIT.
Chitosan and Carboxymethylated short-chain amylose (Ji et al., 2019)	Antisolvent precipitation	Spherical core–shell	Insulin	(1) CS improved oral bioavailability of insulin by increasing tansepithelial permeability via endocytosis and a paracellular route. (2) Nanocomposites had a stronger hypoglycemic by allowing inlulin sustained-release.
Carrageenan (Chen et al., 2020, Cheng & Jones, 2017)	Antisolvent precipitation	Spherical core–shell	Curcumin Piperine	(1) Improved the photochemical and thermal stability of ingredients. (2) Improved oral bioavailability of ingredients by delaying the release in GIT.
Shellac (Sun, Xu, et al., 2017)	Antisolvent co-precipitation	Network structure containing several microspheres or random geomery		(1) Improved the photochemical and thermal stability of ingredients. (2) Provided great sustained release property of curcumin in GIT.
Hydroxypropyl methyl cellulose (Dinh et al., 2017)	Conjugation	Spherical particles	Paclitaxel	Increased the solubility and dissolution rate of a poorly water-soluble drug
Tea polysaccharide (TPS) (Li et al., 2016)	pH-Induced antisolvent precipitation	Spherical core–shell	Paclitaxel	The paclitaxel was encapsulated in an amorphous form and had a typical sustained release in PBS (pH 7.4)
Gum arabic (Chen, Fu, et al., 2019; Li, Xu et al., 2018)	pH-Induced antisolvent precipitation or antisolvent precipitation	Spherical core–shell	Curcumin	(1) pH-Controlled release of curcumin in GI (2) High loading capacity of curcumin. (3) Enhancement of the photostability of curcumin. (4) Increase of pH or temperature companies a faster degradation of curcumin.
Soybean polysaccharide (Li et al., 2019)	pH-Induced antisolvent precipitation	Spherical	Quercetin	(1) The EE was higher than zein particles without coating. (2) The photochemical stability and ABTS + scavenging ability of quercetin were significantly enhanced.
Pectin (Huang et al., 2017; Hu et al., 2015; Huang et al., 2019)	pH-Induced antisolvent precipitation	Spherical core–shell	Curcumin Resveratrol	(1) Higher antioxidant activity in vitro. (2) Higher antiproliferative activity when tested using human hepatocarcinoma. (3) A slight increase of release of ingredients in SGF but a much more rapid release in SIF.
Pectin and alginate (Huang et al., 2016)	pH-Induced antisolvent precipitation	Spherical core–shell	Curcumin	(1) Improved antioxidant activities of curcumin. (2) Improved the water dispersibility and physical stability of curcumin.

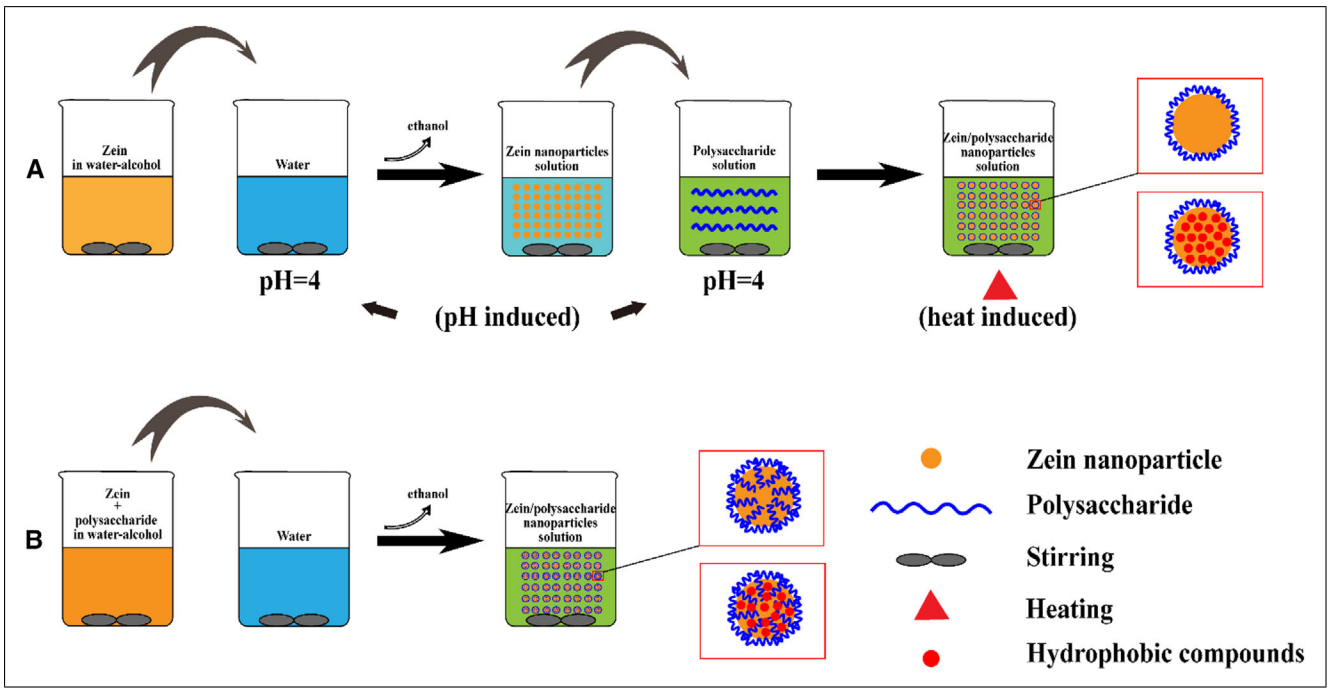

Figure 1–The methods of preparation of zein/polysaccharide nanoparticles: (A) pH- and heat-induced antisolvent precipitation; (B) antisolvent coprecipitation.

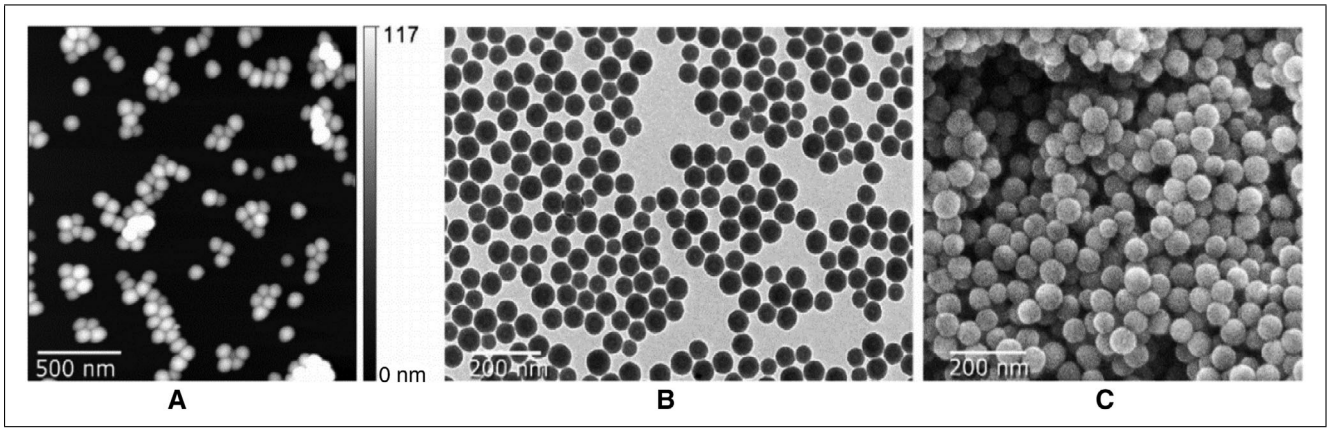

Figure 2–The morphological observation of nanoparticles by (A) AFM, (B) TEM, and (C) SEM. Reprinted from Eaton et al. (2017) with permission from Elsevier.

mixing, the zein nanoparticle solution and the polysaccharide solution are brought to a certain pH and are heated at a certain temperature. The heating treatment can impact the zein core and make smaller nanoparticles (Chang, Wang, Hu, & Luo, 2017; Wang & Zhang, 2019).

Antisolvent co-precipitation (Figure 1B): In this method, both zein and polysaccharides are dissolved into aqueous ethanol together, and the mixture is then poured into water. The subsequent steps are the same as those of the antisolvent precipitation. This preparation is suitable for polysaccharides that are soluble in ethanol. Thus far, only propylene glycol alginate (PGA), shellac and hyaluronan have been used in this procedure (Chen, Han, et al., 2018; Chen, Han, et al., 2019; Sun et al., 2016; Sun, Yang, Dai, Chen, & Gao, 2017; Wei, Yu et al., 2019; Wei, Sun, Dai, Zhan, & Gao, 2018).

3.2 Morphological observation of zein/polysaccharide nanoparticles

Scanning electron microscopy (SEM), atomic force microscopy (AFM), and transmission electron microscopy (TEM) are three common tools to obtain information on nanoparticles (as shown in Figure 2).

SEM allows us to see the surface of any sample and to obtain three-dimensional images. AFM is able to determine the surface of a material on the atomic scale. Both SEM and AFM can provide surface information and show three-dimensional images of a sample, but AFM cannot observe the morphology of polysaccharides alone and is more suitable to resolve subtle changes on a highly smooth surface (Sun, Dai, & Gao, 2017b). TEM can provide information about internal structures of samples by showing two-dimensional images, but it is limited to samples that are thin enough to let electrons pass through.

From the previous research on the morphology of nanoparticles, the following conclusions could be made: SEM is the most suitable tool for microscopically observing zein/polysaccharide nanoparticles, which have a droplet size in the range of 100 to 1000 nm. Eaton et al. showed that SEM was suitable for large nanoparticles (above 50 nm in diameter), while AFM and TEM could provide accurate results with smaller nanoparticles (Eaton et al., 2017).

3.3 Analysis on mechanism of formation of zein/polysaccharide nanoparticles

The fabrication of zein/polysaccharide nanoparticles usually includes two phases: first, zein molecules aggregate into nanoparticles accompanied by the encapsulation of the hydrophobic compounds; second, the zein nanoparticles bind with the polysaccharide molecules to form the core–shell nanoparticles. In addition, some polysaccharides that are soluble in aqueous ethanol can aggregate together with zein into nanoparticles and encapsulate the compounds. During the formation of nanoparticles, a mechanism is required to explain the interactions between the compounds with zein molecules, the interaction forces between zein and polysaccharides, and the state of the compound inside nanoparticles.

Some instrumental analysis methods, such as dynamic light scattering (DLS), zeta potential, fluorescence spectroscopy, Fourier transform infrared spectroscopy (FTIR), circular dichroism spectroscopy (CD), and X-ray diffraction (XRD), have been used to investigate the formation of nanoparticles to provide an explanation of this mechanism.

3.3.1 Dynamic light scattering. DLS not only provides the droplet size of nanoparticles but also the particle size distribution. Previous studies determined that the droplet size of zein nanoparticles could decrease below 100 nm, but when binding with polysaccharides, the droplet size will increase (Hu & Mcclements, 2015; Huang et al., 2017; Khan et al., 2019; Sun, Dai, & Gao, 2017b).

3.3.2 Zeta potential. Zeta potential is a measure of the effective electric charge on the nanoparticle's surface. The higher the magnitude of potential, the more net positive or negative charges on the particle's surface, indicating stronger electrostatic repulsion among particles and, therefore, an increase of stability (Selvamani, 2017). Additionally, zeta potential can help to detect if there are enough polysaccharide molecules to coat the zein nanoparticle's surface to determine the optimal mass ratio of zein to polysaccharide (Li, Xu et al., 2018).

3.3.3 Fluorescence spectroscopy. Fluorescence spectroscopy is used to determine the fluorescence intensity of samples. There are two ways to use it to prove the interactions between zein and polysaccharides or between zein and the compounds encapsulated. The first approach is to test the nanoparticle's surface hydrophobicity (Li, Xu et al., 2018, 2019). In this test, zein/polysaccharide nanoparticles dispersions are mixed with a hydrophobic fluorescent probe [8-anilino-1-naphthalene sulfonate (ANS)], an anion surfactant [sodium dodecyl sulfate (SDS)], and NaCl, after which the fluorescence intensity of ANS is detected. The changes in fluorescence intensity depend on the number of ANS molecules bound to the hydrophobic patches on the surface of nanoparticles. The decrease of the fluorescence intensity with the increase of concentration of polysaccharides indicates that more polysaccharide molecules covered on zein nanoparticle's surface block the ANS from binding to zein. However, the increase of the fluorescence intensity with the increase of concentration of SDS or NaCl suggests that the electrostatic and hydrophobic forces between zein and polysaccharides are disrupted. The results obtained above prove that there are both electrostatic and hydrophobic forces between zein and polysaccharides in the zein/polysaccharides nanoparticles (Li et al., 2019). The second approach is to test the fluorescence intensity of zein before and after interacting with polysaccharides (Chen et al., 2018). Because of the high level of tyrosine residues in zein, the fluorescence is easily detected when zein is excited at 280 nm. The increase of fluorescence intensity indicates a more hydrophobic environment surrounding tyrosine residues, suggesting changes in the structure of zein molecules. Previous researches has proved that the fluorescence intensity of zein increases after forming complexes with polysaccharides, such as hyaluronic acid and sodium hyaluronate (Liu, Jing, Han, Zhang, & Tian, 2019), PGA (Sun et al., 2016; Sun, Dai, & Gao, 2017b; Wei, Yu et al., 2019), chitosan (Ren et al., 2019), and agar (Kaushik, Rawat, Aswal, Kohlbrecher, & Bohidar, 2019). However, after the compounds are encapsulated into nanoparticles, the fluorescence intensity of zein decreases due to fluorescence quenching, suggesting that the interactions occur between zein and compounds (Chen et al., 2020; Sun et al., 2016; Wei, Yu et al., 2019).

3.3.4 Fourier transform infrared spectroscopy. FTIR is commonly used to investigate the interactions in nanoparticles and efficiently identify the functional groups. The vibrations of repeat units of protein produce some characteristic IR absorption bands, namely, amide A, amide B, amide I to VII, and so on (Kong & Yu, 2007). Among them, the amide I and II bands are the most important bands, as they are sensitive to the protein secondary structure. The amide I peak position occurs in the region of 1700 to 1600 cm^{-1} (mainly C=O stretch), and the amide II band occurs in the region of 1600 to 1500 cm^{-1} (C–N stretch coupled with N–H bending mode). The band at 3100 to 3500 cm^{-1} is due to the O–H stretching vibration of the hydroxyl group (Li et al., 2019). The spectrum of CH_2 antisymmetric and symmetric stretching vibrations is in the range of 3000 to 2800 cm^{-1} (Hasni, Bourassa, & Tajmir-Riahi, 2011). The peak shift of the band in 3100 to 3500 cm^{-1} usually suggests the occurrence of hydrogen bonds. The changes of the bands in the amide I and amide II commonly indicate the transformation of the secondary structure of zein as well as the appearance of hydrogen bonds. The band at 3000 and 2800 cm^{-1} may provide the information about the hydrophobic forces.

3.3.5 Circular dichroism spectroscopy. CD is an excellent tool for the rapid determination of the secondary structure and folding properties of proteins (Banerjee et al., 2019). The proportion of secondary structures of a protein can be measured by the CD signal in the far UV region (180 to 250 nm). In previous reports, the CD measurement proved that the α-helix content of zein decreased after binding with polysaccharides, while the β-sheet and β-turn contents increased (Chen et al., 2018, 2020; Sun et al., 2016; Sun, Dai, & Gao, 2017b; Wei, Yu et al., 2019).

3.3.6 X-ray diffraction. XRD is a powerful tool to study crystal materials to obtain information on structure, phases, and other structural parameters (Kohli, 2012; Rajeshkumar, Bharath, & Geetha, 2019). In studies on zein/polysaccharide nanoparticles, XRD has been used to investigate the state of the encapsulated compounds, such as resveratrol (Khan et al., 2019; Wei, Yu et al., 2019), quercetagetin (Sun et al., 2016), quercetin (Li et al., 2019), curcumin (Chen et al., 2020), PTX (Ngo, Tran, Lee, & Tran, 2019), and CoQ10 (Wei, Zhang et al., 2019), which were proved to be in the amorphous form after encapsulation into nanoparticles. Further, the decrease of the peak intensity of zein/polysaccharide complexes indicated the formation of the amorphous complexes, suggesting an intermolecular interaction occurred between zein and polysaccharides (Khan et al., 2019; Li et al., 2019; Sun, Dai, & Gao, 2017a).

Each of these analysis tools discussed above can provide some important information based on their specialty. Putting these pieces of information together and analyzing them can reveal a possible mechanism for the formation of zein/polysaccharide

nanoparticles. Commonly, the aggregation of zein molecules into nanoparticles is driven mainly by hydrophobic forces with changes in zein structure. The interactions between zein and polysaccharides include predominantly hydrogen, hydrophobic, and electrostatic adsorption forces. The level of polysaccharides also induces the zein structure to change. The compounds that are encapsulated into nanoparticles stay in the amorphous state rather than the crystal state. Moreover, the interactions between compounds and zein are predominantly driven by hydrophobic forces, but hydrogen bonds are potentially involved for some substances.

4 DEVELOPMENT OF ZEIN/POLYSACCHARIDE NANOPARTICLES

The efforts to study zein/polysaccharide nanoparticles has focused on four aspects: (i) extension of the application of bioactivities, especially for hydrophobic substances, to make them disperse in water or oil liquid food, solid food, functional food, and food pack; (ii) improvement of the stability of bioactivities to environmental changes, such as pH, ionic strength, thermal treatment, and storage; (iii) enhancement of the bioavailability of bioactivities by controlled release in the gastrointestinal tract (GIT) and decreased cytotoxicity; and (iv) encapsulation efficiency, loading capacity, and active maintainability or improvement of bioactivities. The previous studies have almost confirmed that the zein/polysaccharide nanoparticles were good delivery systems for nutrients, bioactivities, and drugs because they made great progress on the goals discussed above.

4.1 Alginate and propylene glycol alginate

Alginate, an anionic linear polysaccharide, is widely used in the food industry as a stabilizer or as a thickening or emulsifying agent. In the presence of Ca^{2+}, alginate can produce a gel structure, which finds use as a thickening agent in the food industry. The only alginate derivative used in food is PGA, which shows good solubility in aqueous ethanol solution (Wei, Yu et al., 2019). At low and intermediate degrees of esterification, PGA is able to form gels with relative strength but has a poor gelling ability with a high degree of esterification (87.9%). PGA is higher tolerance to Ca^{2+} and is much less sensitive to low pH, conditions under which regular alginate will precipitate (Nilsen-Nygaard, Hattrem, & Draget, 2016; Sun, Chen, Dai, & Gao, 2017).

The fabrication of zein/alginate nanoparticles (ZANs) has commonly utilized the pH-induced antisolvent precipitation process. The incorporation of alginate improved the stability of nanoparticles to pH, ionic strength, and thermal treatment. Additionally, the ZANs protected the encapsulated compounds from harsh conditions, controlled their release in GIT, and enhanced their activities. Using pH-induced antisolvent precipitation, Hu et al. produced ZANs with a core–shell structure with a zein core and an alginate shell. Their results showed that ZANs had good stability from pH 3 to 8 but aggregated at pH 2. The aggregation did not occur when the concentration of NaCl was as high as 100 mM at pH 7 and 2 M at pH 4. The thermal stability proved very good at pH 7 after heating at 90 °C for 120 min (Hu & Mcclements, 2015). These results illustrated that ZANs had potential as a nanodelivery system. Lee et al. studied ZANs as an oral drug delivery system to carry superoxide dismutase (SOD). Their results showed that the ZANs could protect SOD from the harsh conditions of GIT and released it in the small intestine to reduce the intracellular reactive oxygen species level (Lee, Kim, & Park, 2016). Bautista et al. obtained similar results on limonoids encapsulated in ZANs. ZANs enhanced the limonoids encapsulation efficiency and stability, improved their activities, and controlled their release in GIT (Bautista, Vidallon, Salamanez, & Rodriguez, 2019).

Zein/PGA nanoparticles (ZPGANs) could be formed by antisolvent coprecipitation as PGA was soluble in aqueous ethanol. The formation and properties of ZPGANs greatly depended on the mass ratio of zein to PGA and the concentration of Ca^{2+} (Sun, Dai, & Gao, 2017a, 2017b). ZPGANs show a fruit tree-like structure. The zein nanoparticles are the "fruit," which attaches to the PGA "branch," as shown in Figure 3. Electrostatic attraction, hydrogen bonds, and hydrophobic interactions were involved in the formation of ZPGANs (Sun et al., 2016; Sun, Dai, & Gao, 2017a, 2017b). ZPGANs improved the entrapment efficiency and loading capacity of the compounds encapsulated (Sun et al., 2016). The incorporation of Ca^{2+} in ZPGANs resulted in a more compact and dense structure. With the increase of Ca^{2+} concentration, the "branches" began closing to each other and cross-linking to form an interwoven flat structure. Ca^{2+} resulted in the formation of strong hydrogen bonds between zein and PGA, strengthened their hydrophobic interactions (Sun, Chen, Dai, & Gao, 2017). Further, the presence of Ca^{2+} diminished the degradation of ZPGANs by pancreatin and pepsin, resulting in the sustained release of drugs (Sun, Yang, Dai, Chen, & Gao, 2017).

4.2 Pectin

Pectin is an anionic structural polysaccharide from the plant cell wall. It is enriched in blocks of (1 → 4)-linked galacturonic acid and galacturonic acid methyl ester units interrupted by single (1 → 2)-linked rhamnose units. The gelling and thickening properties of pectin strongly depend on the degree of esterification of galacturonic acid (Liu et al., 2006). Pectin can form Ca^{2+}-induced hydrogels, which have been used as a delivery system for colon-specific drugs, as they can stay intact in gastric fluids but release the encapsulated compound in intestinal fluids (Chotiko & Sathivel, 2017).

A pH-induced antisolvent precipitation has been mainly used to fabricate the zein/pectin nanoparticles (ZPNs). Pectin has carboxyl groups with a dissociation constant (pKa) of approximately pH 3.5 and will be strongly negatively charged at pH 4.0 (Huang et al., 2017). Thus, pectin molecules with negative charges can interact with cationic zein nanoparticles (p*I* 5 to 6) to form core–shell complexes through electrostatic adsorption forces. ZPNs can enhance the encapsulated compounds' water-dispersibility and activities. Moreover, ZPNs have good stability to pH and thermal treatment but are unstable at high ionic strength.

Hu et al. incorporated curcumin into core–shell ZPNs by pH-induced antisolvent precipitation to improve curcumin's water dispersibility (Hu et al., 2015). FTIR and Raman spectroscopy measurement indicated that the aromatic rings in curcumin interacted with zein through hydrophobic interactions. In the differential scanning calorimetry profiles, there was no evidence of a sharp endothermic peak around 177 °C , which was the peak of pure curcumin, suggesting that curcumin was in an amorphous form in ZPNs. Huang et al. encapsulated resveratrol into ZPNs in the same way (Huang et al., 2017, 2019). The results showed that encapsulated resveratrol had a good *in vitro* antioxidant and anticancer properties and higher bioaccessiblility than free resveratrol. ZPNs were stable to aggregation over a wide pH range (2 to 7) and thermal processing (80 °C for 1 hr, pH 4) but broke down when exposed to high ionic strengths (>50 mM NaCl, pH 4). However, the presence of alginate improved the stability of nanoparticles to the high ionic strength (Huang et al., 2016). In addition, sodium caseinate (NaCas) as a stabilizer was incorporated into

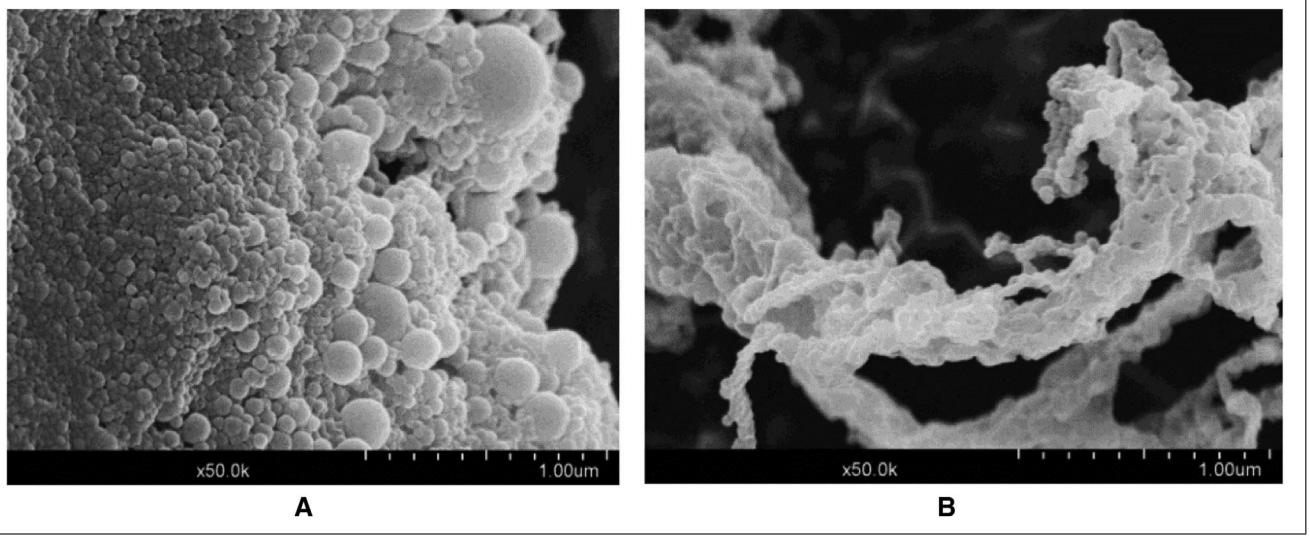

Figure 3–SEM images of (A) zein nanoparticles and (B) zein/PGA nanoparticles. Reprinted from Sun et al. (2016) with permission from Elsevier.

ZPNs to form zein/casein/pectin nanoparticles by both pH- and heat-induced antisolvent precipitation methods (Veneranda et al., 2018). The formation of nanoparticles by heating treatment at an acidic pH close to the p*I* induced partial protein denaturation and enhanced electrostatic interactions between protein and polysaccharides (Chang et al., 2017). The NaCas in the formulation was beneficial for maintaining the original nanoscale dimension during the redispersion.

4.3 Chitosan

Chitosan, the deacetylated product of chitin, consists of units of D-glucosamine and N-acetyl-D-glucosamine, which are linked together by a glycosidic β (1–4) bond. Chitosan is insoluble in water, alkaline solutions, and organic solvents. Conversely, it is soluble in dilute acid solutions below pH 6.3. Chitosan and its derivatives have shown diverse biological activities, including antioxidant, antihypertensive, anti-inflammatory, anticoagulant, antitumoral, antimicrobial, hypocholesterolemic, and antidiabetic effects (Sánchez-Machado et al., 2019). Chitosan has been employed in carriers for the oral administration due to its resistance to acid pH values and the positive charge, which allows an electrostatic interaction with the negative charge of the sialic acid present in the intestinal mucus, thus resulting in improved bioavailability after oral administration (Pauluk, Padilha, Khalil, & Mainardes, 2019).

In previous studies, bioactives such as α-tocopherol, curcumin, resveratrol, and astaxanthin were incorporated into nanoparticles based on zein and chitosan (Ji et al., 2019; Li, Chen et al., 2018; Luo, Zhang, Whent, Yu, & Wang, 2011; Pauluk et al., 2019). Antisolvent precipitation was mainly used in the fabrication of the nanoparticles without pH or heat induction. Zein/chitosan nanoparticles (ZCNs) were proven to have the capacity to protect encapsulated compounds and control their release in GIT. The electrostatic interactions and hydrogen bonds are major forces responsible for ZCNs formation. However, because chitosan is poorly soluble in water, it was considered to be replaced by oligochitosan to prepare zein/oligochitosan nanoparticles (ZOCNs) (Jiang & Zhu, 2019). ZOCNs provided astaxanthin a good dispersion in liquid food and improved its stabilities upon exposure to UV light and storage. In addition, ZCNs were found to be sensitive to pH; therefore, alginate was employed to overcome this defect through the formation of zein/alginate/chitosan nanoparticles (Khan et al., 2019; Li, Chen et al., 2018).

4.4 Other polysaccharides

4.4.1 *ι*-Carrageenan. Carrageenan is a group of water-soluble anionic sulphated polysaccharides. There are three main varieties of carrageenan: kappa-carrageenan, iota-carrageenan, and lambda-carrageenan, among which *ι*-carrageenan is widely used in the food industry (Yang & Yang, 2020b). Because of the sulfate groups (p*K*a ~2), carrageenan has a relatively high charge density. The extraction and application of carrageenan were deeply studied (Sow, Nicole Chong, Liao, & Yang, 2018; Yang & Yang, 2020a; Yang, Gao, & Yang, 2020). The results showed that the gelation of carrageenan was affected by its forms, and that protein-carrageenan interaction also be influenced by carrageenan forms (Feng et al., 2020). It could potentially stabilize protein nanoparticles over a wider pH range (Cheng & Jones, 2017).

Core–shell zein/carrageenan nanoparticles were made by the antisolvent precipitation method. The presence of carrageenan significantly enhanced the stability of zein nanoparticles to pH and storage (Cheng & Jones, 2017). Ca^{2+} acted as a cross-linker for the outer layer to form a salt bridge between anionic carrageenan molecules. Electrostatic attraction, hydrogen bonding, and hydrophobic interactions participated in the formation of the nanoparticles. Chen et al. (2020) found that zein/carrageenan nanoparticles effectively retarded the photo- and thermal-degradation of the encapsulated curcumin and piperine and delayed the release of nutrients under *in vitro* gastrointestinal conditions.

4.4.2 Hydroxypropyl methylcellulose. Hydroxypropyl methylcellulose (HPMC) is a nonionic water-soluble polymer that is derived from cellulose. Its swelling and dissolution properties in aqueous medium make it an excellent vehicle to control the drug release (Notario-Pérez et al., 2019).

Zein/HPMC nanoparticles (ZHNs) can be made by using both antisolvent and conjugation approaches (Dinh et al., 2017; Ngo et al., 2019). Ngo et al. found that ZHNs enhanced the dissolution rate of a poorly water-soluble drug. Moreover, the presence of a surfactant could further improve the wettability, leading to the minimization of drug crystals. When ZHNs were formed by

conjugation, the reactions occurred between the –COO groups in zein and the –OH groups in HPMC.

4.4.3 Tea polysaccharides. Tea polysaccharides (TPs), extracted from green tea, are protein-bounded acidic polysaccharides. Research has shown that zein/TPs nanoparticles sustain the release of encapsulated compounds and keep the compounds in an amorphous state (Li et al., 2016).

4.4.4 Gum arabic. Gum arabic (GA) has good solubility over a wide range of pH and good emulsifying properties due to the polypeptide glycated on the saccharide chain. Zein/GA nanoparticles (ZGNs) have good stability at pH 3 to 9, but thermal treatment (more than 70 °C) could affect the structure of ZGNs, resulting in the leakage or exposure of encapsulated compounds (Chen, Fu, et al., 2019; Li, Xu et al., 2018). In addition, Chen et al. found that Fe^{3+} could penetrate the nanoparticles and decrease the photostability of curcumin.

4.4.5 Hyaluronic acid. Hyaluronic acid (HA) consists of D-glucuronic acid and *N*-acetyl-glucosamine. It is biocompatible with many physiological functions, such as antiaging, antiinflammation, angiogenesis, and embryonic development. Because HA is able to dissolve in aqueous ethanol, zein/HA nanoparticles can be fabricated by the antisolvent coprecipitation (Chen et al., 2018; Chen, Han, et al., 2019). Electrostatic attraction was the dominant driving force between zein and HA, followed by hydrogen bonding and hydrophobic effects. The incorporation of HA led to a conformational change of zein and improved zein's physical and thermal stability. Zein/HA nanoparticles enhanced the stability of encapsulated compounds and controlled their release in GIT.

4.4.6 Rhamnolipids. Rhamnolipids are surface-active glycolipids that have one or two hydrophilic rhamnose units attached to a hydrophobic fatty acid chain. Previous studies have shown that rhamnolipids could improve the aggregation stability of both organic and inorganic nanoparticles.

pH-induced antisolvent precipitation has been used to produce zein/rhamnolipids nanoparticles (ZRNs) encapsulating curcumin (Wang et al., 2019). The mixture of zein and curcumin was initially prepared in water rather than water–alcohol as the pH was adjusted to 12. This method was used because both zein and curcumin can dissolve in a strongly alkaline solution. When the rhamnolipids solution was blended with alkaline solution, the pH was adjusted to neutral, after which the nanoparticles formed. The research showed that ZRNs were stable from pH 5 to 9. At neutral pH, ZRNs were stable at low ionic strengths (<100 mM NaCl) and low temperatures (37 or 55 °C). The nanoparticles could protect curcumin from degradation during 1 month of storage at both 25 and 37 °C.

4.4.7 Chondroitin sulfate. Chondroitin sulfate (CS) is a linear polysaccharide with repeating disaccharide units of (1–3)-β-*N*-acetyl-*d*-galactosamine and (1–4)-β-glucuronic acid. Each monosaccharide might have between zero and two sulfate groups, thus providing an overall negative charge (dos Santos & Grenha, 2015).

Zein/CS nanoparticles have a regular spherical structure (Yuan et al., 2019). Electrostatic interactions, hydrogen bonding, and hydrophobic interactions participated in the formation of the nanoparticles. The nanoparticles have great stability to pH and heat treatment, but the stability to the ionic strength depends on the pH values. At pH 4 the nanoparticles are stable in the ionic strength range of 0 to 15 mM and are stable within the range of 0 to 10 mM at pH 7.

4.4.8 Soybean polysaccharide. Soybean polysaccharide (SP) is a by-product obtained during the production of tofu and soybean protein. It is a highly water-soluble, low-viscosity, heat-stable, and negatively charged polysaccharide. The polysaccharide also contains a small amount of hydrophobic protein that contributes to its interfacial activity.

Zein/SP nanoparticles (ZSNs) have been proven to be stable at pH 2 to 8 and high temperature (Li et al., 2019). However, the nanoparticles exhibited different ionic strength stabilities at pH 4 and 7. Quercetin, a natural flavonol with many activities, can be encapsulated into ZSNs, which enhanced its encapsulation efficiency, photochemical stability, and $ABTS^{+}$ scavenging ability.

4.4.9 Agar. Agar, a structural carbohydrate in the cell walls of agarophytes algae, is easily soluble in hot water. Due to its extraordinary gelation properties and exceptional saturation limit, agar has been utilized for many food processing and biomedical applications.

The nanoparticles, also called coacervates, are made up of zein and agar (Kaushik et al., 2019). Small angle neutron scattering has been used to investigate the microscopic structures of the nanoparticles. The nanoparticles prepared in low ionic strength solutions reveal a higher storage modulus and network density but a lower melting temperature. At higher ionic strength, the opposite behavior is noticed. The microstructural changes of the nanoparticles are affected by the mixing ratio of zein to agar.

5 STABILITY OF ZEIN/POLYSACCHARIDE AND CONTROLLED RELEASE OF ACTIVE INGREDIENTS

Previous studies indicated that in the formation of core–shell zein/polysaccharides nanocomplexes, electrostatic attraction, hydrogen bond, and hydrophobic interaction were involved. All or some of them were proved to be responsible for the interaction between zein and polysaacharides. When zein nanoparticles were covered with enough polysaccharide molecules, the ζ- potential of nanocomlexes was more negative or positive. Then a strong electric repulsion was produced between nanoparticles and prevented nanoparticles from aggregating over a wide pH range or at high ionic strength. Studies showed that the mass ratio of zein to polysaccharides had significant effect on the droplet size of nanoparticles. When the proportion of a polysaccharide was too low, there were not enough polysaccharide molecules to cover the surface of zein nanoparticles. The surface charges were neutralized and the repulsion force between nanoparticles decreased, resulting in the nanoparticles aggregated. With the increasing proportion of polysaccharides the droplet size of nanoparticles decreased until the surface of nanoparticles was saturated. In the meanwhile, previous researches showed that the thermal stability of nanoparticles was improved by coating polysaccharides. This is because that the elevated temperature could enhance the hydrophobic interactions between zein and polysaccharides. But Chein et al. showed that the high temperature could change the nanostructure and resulted in a faster degradation of curcumin (Chen, Fu, et al., 2019).

As shown in Table 1, recent studies indicated that zein/polysaccharides nanoparticles exhibited controlled release of encapsulated active ingredient. The release was more rapid in SIF than that in SGF. This probably occurred because of (i) the bile salts and peptides hydrolyzed from zein molecules, which formed micelles or complexes that solubilized the released ingredients; (ii) the pH could change the secondary and tertiary structure of zein and lead to the controlled release of active ingredients; (iii) the pH could affect the polysaccharide charge density that changed the structure of "shell." When pH increased, anion polysaccharide molecules were more negative and the repulsion force between polysaccharide molecules increased resulting in the

"shell" became loose. This may explain that why the concentration of Ca^{2+} could affect the release of active ingredients. In addition, a heat- and pH-induced nanocomplex could produce smaller and homogeneous nanoparticles, but its effect on the encapsulated active ingredients has not been reported.

6 CONCLUSIONS

The advantages of encapsulation of the compounds into zein/polysaccharides nanoparticles are exhibited as follows: (i) high encapsulation efficiency and loading capacity; (ii) good dispersion in water; (iii) improvement of stability to harsh conditions; (iv) control release and enhancement of bioavailability; and (v) maintaining or enhancement of activities. Moreover, zein and polysaccharides are food materials and easy to obtained. The preparation of nanoparticles need not use synthetic additives and toxic organic solvents. So it would be economic, environmentally friendly to utilize zein/polysaccharides nanoparticles. In the presence of the polysaccharides, zein nanoparticles obtain improvements in stability to pH, ionic strength, and thermal treatment, but the stability significantly depends on the types and levels of polysaccharides. Moreover, high ionic strength and high temperature still pose a challenge in the application of zein/polysaccharides nanoparticles. Some previous studies have shown that the encapsulation of compounds into nanoparticles enhanced their activities, but investigations into the mechanism have not been carried out. Further, the bioavailable experiments were performed almost entirely *in vitro*; therefore, the pharmacokinetics *in vivo* should be further studied in the future.

AUTHOR CONTRIBUTIONS

Li Ming wrote the manuscript, and Yu Meihui contributed to the collection of information of polysaccharides.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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