

Recent Advances in Zein-Core/Polysaccharide-Shell Nanoparticles (Alginate, Pectin, Chitosan, Carrageenan and Hyaluronic Acid) for Bioactive Compound Delivery: Preparation, Characterization, Stability, and Release

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Maize prolamins are proteins that, when isolated, can form spherical nanoparticles suitable for delivering hydrophobic bioactive molecules via self-assembly. However, at physiological pH, zein nanoparticles readily aggregate and are susceptible to degradation in the gastrointestinal (GIT) tract by proteases. These limitations can be circumvented by wrapping zein cores with polysaccharide shells that impart electrostatic and steric stabilization and allow controlled, stimulus-responsive release of encapsulated compounds. The physicochemical properties of zein, methods used to prepare zein/polysaccharide nanoparticles (antisolvent precipitation, pH-induced, heat-induced and antisolvent co-precipitation) their morphological characterization using scanning electron microscopy (SEM), atomic force microscopy (AFM) and transmission electron microscopy (TEM), and mechanistic interactions were revealed using dynamic light scattering (DLS), zeta potential, fluorescence spectroscopy, Fourier transform infrared (FTIR), circular dichroism (CD) and X-ray diffraction (XRD) studies were taken in the studies analyzed in this descriptive literature review and compiled between the years 2015 – 2026. The controlled release and stability of typical zein/polysaccharide systems such as alginate, pectin, chitosan, carrageenan, hyaluronic acid, gum Arabic, soybean polysaccharide, and chondroitin sulfate are reviewed. Several knowledge gaps remain, including interbatch reproducibility, manufacturing scalability, in vivo bioavailability, and the molecular mechanisms underlying increased biological activity. By addressing these challenges, these natural and food-safe nanocarriers show strong potential for nutraceutical, pharmaceutical, and functional food applications.

Keywords: Bioavailability, Controlled Release, Core-Shell Nanoparticles, Encapsulation Efficiency, Food Nanotechnology, Narrative Review, Polysaccharide Coating, Zein.

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The nutritional and functional quality of foods continues to improve rapidly; however, many bioactive compounds, such as α -tocopherol, resveratrol, and curcumin, are highly water-insoluble and susceptible to degradation under pH changes, heat, and light. These physicochemical constraints significantly diminish their nutritional and functional potential and limit their bioavailability. Such compounds can be encapsulated in nano-delivery systems that incorporate them into biodegradable, biocompatible matrices to protect them from degradation, enhance aqueous dispersibility, and enable controlled release in the gastrointestinal tract.^{1,2}

Zein, a large storage protein from maize, has received increasing attention as a nanoparticle matrix owing to its high-hydrophobicity amino acid composition,^{3,4} which enables spontaneous self-assembly into spherical nanoparticles upon transfer from ethanol-water solutions into aqueous environments. This process, referred to as antisolvent precipitation, is scalable, simple, and does not require toxic reagents, making it attractive for food-grade applications. However, pure zein nanoparticles suffer from significant limitations: they readily aggregate at their isoelectric point (pI H^+ 6.2) and at high ionic strengths, and they are readily degraded by pepsin in the stomach.^{1,3,8-13}

Such polysaccharides as chitosan, alginate, pectin, carrageenan, hyaluronic acid are widely used in the food industry due to their biocompatibility, biodegradability and functional versatility. Electrostatically, these polysaccharides are capable of binding onto zein nanoparticle surfaces with their ionizable carboxyl, hydroxyl, or amino groups, with the resulting core-shell nanocomplexes having greater colloidal stability and stimulus-responsive release. The zein/polysaccharides blends also widen the scope of the hydrophobic bioactives that can be effectively encapsulated when compared with zein alone.⁵

The zein/polysaccharide nanocompartment is an integrated core-shell system, and although previous studies have explored the zein-based delivery system in the form of films, fibers, and emulsions, few have explored this core-shell system.^{1,3,4,6} This narrative review aims to fill that gap by summarizing the existing information on zein properties, zein/polysaccharide nanoparticle formation and morphology, stability in different

pH and ionic strength conditions, controlled-release properties as a function of polysaccharide types and future research needs.^{1,3,5}

METHODOLOGY

The present review is a narrative type synthesis based on the reviewed literature published between 2015 and 2026 on zein nanoparticles and its systems based on the use of polysaccharide coating. The scope includes zein composition, fractionation, and physicochemical properties; nanoparticle fabrication strategies; morphological and structural characterization methods; release kinetics and stimuli-responsive mechanisms; future research directions; and outstanding challenges related to polysaccharide-specific systems and their stability properties. Representative studies were selected to illustrate key concepts and compare results across systems. This method allows for an integrated analysis of various fabrication techniques, characterization techniques and biological results.

Characteristics of zein

Composition and Fractionation

Zein comprises approximately half of the total protein in whole-grain maize and is typically derived from corn distillation by-products, such as corn gluten meal. Zein is classified as Generally Recognized as Safe (GRAS) by the US FDA. Zein is a heterogeneous mixture of homologous proteins grouped into four main subfractions based on solubility and molecular weight. The most abundant fraction of zein is α -zein, which comprises 75–85% of total zein and can be extracted with 50–95% 2-propanol from endosperm tissue. The least abundant fraction is $\bar{\alpha}$ - and $\bar{\alpha}$ -zeins, composing 10–15% of total zeins, which can be extracted with aqueous 2-propanol solutions and aqueous solutions of ethanol. The relative abundance of these fractions varies with maize source and extraction method, thereby influencing nanoparticle formation kinetics and final particle properties.⁸⁻¹⁰

Physicochemical Properties

Zein is insoluble in pure water but dissolves in aqueous alcohols, high-urea solutions, strongly alkaline media (pH > 11), and anionic surfactant solutions. More than half of its amino acid residues are hydrophobic, conferring pronounced

amphiphilicity. Small-angle X-ray scattering data reveal that zein adopts a conformation of nine to ten antiparallel amphiphilic α -helical segments connected by turns rich in glutamine. The hydrophilic terminal loops and hydrophobic helical faces cooperatively promote self-assembly and formation of films, fibers, and nanostructures.^{8,11-13}

The isoelectric point (pI) of zein is about 6.2, but it varies depending on the purity of zein, its source, solvent composition, and reaction conditions. When the pH is below the pI, zein has a net positive charge; when it is above the pI, it has a net negative charge. The electrostatic interaction that governs pH-dependent charge behavior drives polysaccharide coating.

Preparation of Zein Nanocarriers

The predominant fabrication strategy is antisolvent precipitation. Zein dissolved in 70–90% aqueous ethanol is added to water; the resulting change in solvent polarity triggers hydrophobic collapse of the α -helices, initiating rapid nucleation and nanoparticle formation while simultaneously entrapping dissolved hydrophobic cargo. The shear rate, ethanol concentration, and zein concentration all influence the final particle diameter. Alternative methods include electrospraying, electrospraying, and supercritical antisolvent co-precipitation, though these require specialized equipment. More recent approaches- flash nanoprecipitation (FNP), permitting controllable particle sizes of 50–108 nm with narrow size distributions, and microfluidic fabrication, offering enhanced control over mixing conditions and particle uniformity- show considerable promise for industrial applications. Regardless of fabrication approach, zein nanoparticles are characterized by smooth surfaces, excellent hydrophobic cores, and low oxygen permeability, collectively protecting encapsulated bioactives against environmental degradation.¹²⁻¹⁸

Applications of Zein-Based Delivery Systems

A diverse range of compounds has been successfully encapsulated in zein nanoparticles, including therapeutic drugs (paclitaxel, ciprofloxacin), nutrients (vitamin D, fish oil, curcumin), polyphenols (resveratrol, quercetin, epigallocatechin gallate), and essential oils. Encapsulation typically enhances aqueous dispersibility, chemical stability, bioaccessibility, and biological activity relative to free compounds.

Zein-based edible coatings applied to fruits and vegetables reduce moisture loss, slow ripening, and inhibit microbial proliferation. Nevertheless, aggregation near the pI and rapid pepsin-mediated digestion in the stomach remain significant limitations that are effectively overcome by polysaccharide coating.^{1,3,15,16}

Zein/Polysaccharide Nanoparticles

Unlike conventional composite and matrix-type particles, a zein/polysaccharide core-shell nanoparticle (CSNP) is a two-compartment particle in which the lipophilic guest resides in the compact hydrophobic zein core, while the polysaccharide corona is at the particle surface and remains hydrated and ionized. The zein core, which is formed by the hydrophobic collapse of amphiphilic alpha-helical sequences, is usually 60-250 nm in diameter, while the polysaccharide coating can contribute about 5-40 nm to the hydrodynamic diameter depending on the molecular weight, charge, and chain conformation. In a true CSNP, the protein and polysaccharide are not uniformly distributed but are distributed into separate core and shell parts of the particle.

Shell formation is facilitated by the interplay of four forces, none of which involves formal covalent bond formation. Net electrostatic interaction between the charge on the zein surface and any net charge-counteracted ionizable functionalities associated with the polysaccharide (i.e., the carboxyl, sulfate, or protonated amino functions) often is the main force behind shell complex formation. This attraction is often augmented by favourable entropic effects arising from displacement of bound counterions and ordered water molecules upon complexation.

Polysaccharide – zein binding via hydrogen bonding occurring at zein turn and loop areas-often the glutamine- and proline-rich terminal segment regions-and polysaccharide backbone hydroxyl moieties is often observed.

When part of the polysaccharide chain of such compounds has lipophilic groups, as in high-methoxyl pectin, partially acetylated chitin, and propylene glycol alginate, hydrophobic forces also contribute. Extended and hydrated macromolecular chains provide steric stabilization. The enthalpy for assembly of complexes is typically not large, and the equilibrium constant is larger than unity, so assembly is spontaneous under appropriate solution

conditions, even if the complex dissociates if the conditions change.

The pH operating window is defined by the interaction between the isoelectric point (pI H⁺ 6.2) and the pKa of an ionizable group on a polysaccharide. In the case of negatively charged polysaccharides (alginate pKa 3.4–3.7; pectin pKa H⁺ 3.5; carrageenan sulfate pKa < 2; hyaluronic acid pKa H⁺ 3.0), the widest attraction window is between approximately pH 3.5 and 5.5 when zein is cationic and the polysaccharide is largely desorbed; the recurring appearance of a depositional procedure at pH 4 in many published examples is hence unsurprising. Cationic chitosan (pKa H⁺ 6.5) needs zein to be negatively charged.

To achieve this, assembly either occurs at pH > pI(zein) or uses the small negatively charged areas which are believed to exist on the heterogeneous zein molecule at slightly acidic pH values.

Ternary system attempts to bridge this gap by inserting a negatively charged intermediary polysaccharide layer between zein and chitosan.

This core-shell structure is primarily stabilized electrostatically. The adsorbed layer increases the magnitude of zeta potential and shifts the point of zero charge away from physiological pH, thereby affecting the aggregation window of bare zein. In addition to steric protection against charge screening, the hydrated chains offer protection at moderate ionic strength. The same layer can also serve as a diffusive barrier and prevent the adsorption of pepsin in the stomach, which will help coated zein particles to continue carrying cargo through the stomach and deliver it to the small intestine.

Preparation Methods

Covalent coupling of zein with polysaccharides (via Maillard reaction or chemical crosslinking) is feasible; however, non-covalent assembly is preferable for food applications because it avoids toxic reagents and reduces processing complexity. Four key non-covalent approaches have been developed¹⁷⁻²⁰:

(1) Antisolvent Precipitation (Route A). Add zein dissolved in 70–90% ethanol to water or an aqueous buffer to generate zein nanoparticles, then add a polysaccharide solution. The polysaccharide molecules adsorb onto the charged zein surface through electrostatic attraction, hydrogen bonding,

and hydrophobic interactions, creating a protective core-shell architecture.

(2) pH-Induced Antisolvent Precipitation (Route A Variant). Adjust both zein and polysaccharide solutions to the same pH (typically 4.0) before mixing. At pH 4.0, zein nanoparticles carry a positive charge (pI 5–6), while anionic polysaccharides carry strong negative charges, maximizing the electrostatic driving force for shell formation.

(3) Heat-Induced Antisolvent Precipitation (Route A Variant). This modification applies thermal treatment following pH adjustment. The zein core is partially opened during heat treatment, yielding smaller, more homogeneous particles with improved interfacial interactions between zein and polysaccharide shell.

(4) Antisolvent Co-Precipitation (Route B). This approach is employed when the polysaccharide (e.g., propylene glycol alginate, hyaluronic acid) is soluble in aqueous ethanol. Zein and the polysaccharide are both dissolved in ethanol and poured simultaneously into water to form intimate composite nanoparticles in a single step.

Morphological Characterization

The morphology of zein/polysaccharide nanoparticles is characterized using three complementary microscopy techniques:

Scanning Electron Microscopy (SEM). SEM produces two-dimensional surface images with topographic contrast and is applicable to nanoparticles in the 100–1000 nm size range to assess sphericity, surface smoothness, and aggregation status. Disadvantages: Dehydration artifacts, particle aggregation due to drying of the sample, differences between the diameters measured by SEM and dynamic light scattering (hydrodynamic diameter) and changes in particle shape in the vacuum of the SEM (but it is important to note that 2D SEM produces an image displaying contrast only in top-down direction and not really a 3D image).^{3,17}

Atomic Force Microscopy (AFM). AFM offers atomic-scale topographic resolution and has been found useful for particles less than 50 nm but can also be used to larger nanoparticles. AFM provides height maps and surface-roughness data without sample staining but cannot reveal internal structure. Important considerations include the

potential for lateral dimension overestimation due to tip convolution, while height measurements are typically more reliable.

Transmission Electron Microscopy (TEM). Transmission electron microscopy (TEM) provides a two-dimensional image of the core internal structure and is typically used to visualize core-shell nanostructures. Image contrast between the shell and core is dictated by differences in electron density, staining treatment, shell material composition, dry-sample preparation, and microscope imaging parameters. Although a light-dark ring around the core may appear, it is not sufficient proof of a shell in polysaccharide nanoparticles; it may reflect partial over-staining of the core or arise from a composition gradient across the nanoparticle volume. If the results are interpreted rigorously and confirmed with another technique, such as zeta potential, dynamic light scattering, or spectroscopic methods, they would be more robust.^{3,17-20}

In practice, SEM is routinely used to characterize size and shape, AFM to examine surface properties at finer scales, and TEM to verify core-shell structure.

Formation Mechanism

The zein/polysaccharide nanoparticles assemble through two consecutive steps. In the initial phase, antisolvent addition-induced polarity changes trigger hydrophobic collapse of the α -helices, leading to rapid nanoparticle nucleation and simultaneous entrapment of hydrophobic cargo. During the second step, polysaccharide molecules adsorb onto the surface of nascent zein nanoparticles via electrostatic attraction, hydrogen bonding, and hydrophobic interactions, forming a protective shell. Certain polysaccharides soluble in aqueous ethanol can co-aggregate with zein during the initial step, resulting in more intimately mixed composite nanoparticles.

The two processes occur on greatly different time scales, and this disparity defines the quality of the obtained shell. Nucleation of zein and subsequent hydrophobic collapse occurs almost fully in a few milliseconds during solution exchange, while adsorption of polysaccharide occurs in seconds to minutes and becomes restricted due to chain diffusion and macromolecular readjustment at the interface. Slow mixing may lead to accumulation of locally supersaturated

volumes, which in turn results in a wide size distribution and partially coated particles.

Use of geometries with better mixing, such as confined impingement jets or microfluidic devices, can overcome this problem

By the same token, placing the polysaccharide before the zein solution meets with the aqueous phase results in co-precipitation, leading to the formation of matrix-like structures rather than discrete shells- which is why not only the final chemical content but also the addition order and rates become so critical. Shell coverage develops with the polysaccharide-to-zein mass ratio; experimentally, it can be described by three zones. Initially uncovered surface - partial coverage causes bridges between particles, producing bridging flocculation. Plateau - saturation coverage in which zeta potential is no longer changing; the hydrodynamic diameter becomes constant.

Surplus - unattached molecules go into the continuous phase, causing depletion flocculation

The saturation ratio, normally ranging from 1:1 to 1:4 zein-to-polysaccharide mass ratio (which may vary, so this depends on the particular system), is best determined by titration and monitored by zeta potential, hydrodynamic diameter, and polydispersity simultaneously. A measure of shell thickness may be found by observing the total increase in hydrodynamic diameter at saturation, but this number should be used with caution, since this accounts for the hydrate shell in particular.

Physicochemical Characterization Methods

Dynamic Light Scattering (DLS) and Zeta Potential

Fluorescence Spectroscopy

DLS reveals that the hydrodynamic diameter increases upon polysaccharide coating, consistent with shell deposition. Bare zein nanoparticles typically range from 50 to 500 nm in hydrodynamic diameter. Surface charge monitoring via zeta potential demonstrates a gradual transition from positive (bare zein at pH 4, typically +20 to +30 mV) to the charge polarity of the polysaccharide shell, providing evidence of coating. The plateau in the zein-to-polysaccharide mass ratio indicates surface saturation. Good DLS interpretation also covers intensity-, volume-, and number-weighted size distributions, as well as polydispersity index, dilution conditions, viscosity

Table 1. Representative zein/polysaccharide core-shell delivery systems, with quantitative parameters (particle size, zeta potential, encapsulation efficiency) and the principal biological outcomes reported for each system *in vitro*

Polysaccharide	Preparation Method	Particle Size (nm)	Zeta Potential (mV)	Encapsulated Compound	EE (%)	Principal Biological Outcome
Alginate/PGA	pH-induced/Co-precipitation	150-300	+25 to -35	Resveratrol, curcumin, SOD	70-85	Enhanced antioxidant activity, GIT stability
Pectin	pH-induced precipitation	120-250	+20 to -30	Curcumin, resveratrol	65-80	Reduced IC ₅₀ in hepatocarcinoma cells
Chitosan	Antisolvent precipitation	100-200	+30 to +45	Curcumin, resveratrol	60-90	Sustained release, mucoadhesion
Carrageenan	Antisolvent precipitation	130-280	+15 to -40	Curcumin, piperine	70-82	Photo/thermal stability
Hyaluronic Acid	Co-precipitation	110-220	+20 to -30	Naringenin, curcumin	>80	Enhanced antioxidant activity
Gum Arabic	pH-induced precipitation	150-300	+10 to -25	Curcumin, tocopherol	60-75	Broad pH stability (3-9)
Soybean Polysaccharide	pH-induced precipitation	100-180	+15 to -35	Quercetin	70-85	High photostability
Chondroitin Sulfate	Antisolvent precipitation	80-200	+25 to -35	Curcumin	65-80	pH/heat stable

and refractive-index approximations, potential multiple-scattering effects, and awareness that hydrodynamic diameter is not necessarily the same as dry-state microscopy measurements.¹⁷⁻²⁰

Fourier Transform Infrared Spectroscopy (FTIR)

The changes in the secondary structure of zein upon polysaccharide complexation are monitored by monitoring the amide I (1600-1700 cm⁻¹, C=O stretching) and amide II (1500-1600 cm⁻¹, C-N stretching bound with N-H bending) bands. Appropriate controls and evaluation of band-width changes and/or peak-position changes, with the assistance of amide I deconvolution where necessary, are necessary for rigorous FTIR interpretation. Changes in the O-H region (3100-3500 cm⁻¹) should be viewed with great caution and should be supported by other evidence.²¹⁻²⁴

Circular Dichroism (CD) Spectrophotometry

Determining secondary-structure content via CD spectroscopy in the far-UV region (180-250 nm) faces challenges specific to nanoparticle dispersions. Since absorption and light scattering may be significant in the far-UV region, a difficult problem with suspensions, the assignment of secondary structure will be impossible in dispersions of the nanoparticle in the far-UV spectral range. Including solvent and baseline subtractions should help, but experiment design must ensure a clean spectral output from opaque dispersions. Reduction in α -helix content and increases in β -sheet and β -turn content upon polysaccharide interaction with zein reflect conformational reorganization associated with nanoparticle assembly, though turbidity-related artifacts necessitate careful control.

X-ray Diffraction (XRD)

XRD reveals that crystalline bioactives (resveratrol, curcumin, quercetin, coenzyme Q10, paclitaxel) become amorphous after nanoparticle encapsulation, typically enhancing dissolution rates. However, the absence of diffraction peaks does not necessarily mean the cargo has been amorphized; other causes include low loading or dilution, small crystallite size, or overlap with the polymer background, which can reduce peak intensity. Dissolution, release, and DSC studies should also confirm this characterization. Decreased crystallinity of zein/polysaccharide complexes evidences close intermolecular interactions.²¹⁻²⁴

Complementary Characterization Methods

The literature increasingly incorporates complementary techniques, including Raman spectroscopy (zein secondary structure, polysaccharide interactions, cargo crystallinity), UV-visible absorption and photostability measurements (photostable compounds), cryogenic TEM (hydrated structures), and small-angle X-ray or neutron scattering (ensemble-level structure).

Analytical Approaches to Exclude

The values of band gaps for zein/polysaccharide delivery nanoparticles and analysis of phonon energies have nothing to do with these polymer complexes, since they are not inorganic semiconductors. That kind of investigation is not meaningful. Here, the reflection of the Raman bands does not correspond to the modes of the phonon crystal, but is rather related to the molecular vibrations.

Encapsulation Efficiency and Loading Capacity

Reporting nanoparticle performance clearly requires including both encapsulation efficiency and loading capacity: $EE(\%) = (\text{mass of encapsulated cargo} / \text{total mass of cargo added}) \times 100$, $LC(\%) = (\text{mass of encapsulated cargo} / \text{mass of nanoparticles}) \times 100$. Many variables influence EE and LC, including cargo polarity; zein and

polysaccharide concentration; solvent ratio; mixing speed; and order of addition.

Structural Interpretation of Indirect Evidence

Surface modification, as demonstrated by charge reversal after the addition of polysaccharide, is not direct proof of a complete and homogeneous core-shell structure, but rather is an indication of surface modification. Indirect evidence should be distinguished from direct structural evidence obtained by TEM or other appropriate methods such as DLS and spectroscopy. The best evidences for a real core-shell structure are obtained by several independent analytical approaches.

Architectural Variants: Binary, Ternary, Layer-by-Layer and Cross-Linked Shells

Zein/polysaccharide binary single-coated capsules are the easiest and most consistent to make and therefore the optimum when scalability and ease of regulation are desired. Introducing a third biopolymer, invariably sodium caseinate, another polysaccharide, or other protein with surfactant properties to zein/polysaccharide delivery systems can increase interface coverage, enable easy redispersion after drying, and enhance a wider range of pH and ionic strength stabilities (e.g. Zein/caseinate/pectin and zein/pectin/alginate based particles).

Table 2. Critical formulation and process variables governing the assembly of zein-core/polysaccharide-shell nanoparticles

Variable	Typical range reported	Principal effect on the core-shell particle
Assembly pH	3.5-5.5 for anionic shells; >6.2 for cationic shells	Controls partner charge and electrostatic deposition
Polysaccharide:zein ratio	1:1-4:1 (w/w)	Controls coverage; too little promotes bridging, excess may cause depletion flocculation
Polysaccharide properties	10-500 kDa; low-to-high charge density	Influence shell thickness, uniformity, mucoadhesion, and salt tolerance
Zein/ethanol feed	5-20 mg/mL zein; 70-90% ethanol	Controls core size, nucleation, polydispersity, and cargo entrapment
Mixing and addition	Stirring to microfluidic/ impinging-jet; sequential or simultaneous	Controls nucleation uniformity and shell versus matrix architecture
Ionic conditions	0-100 mM NaCl during assembly	Charge screening may suppress adsorption and promote aggregation
Ca ²⁺ cross-linking	0-20 mM	Densifies anionic shells and improves salt, enzyme, and release resistance
Solvent removal	Evaporation, dialysis, or ultrafiltration	Affects particle integrity, storage stability, and residual-solvent safety

Alternatively, a third component, added by Layer-by-Layer assembly (depositing alternating charged polyelectrolytes), to zein/polysaccharide particles provides additional benefits of increased flexibility in controlling shell thickness, charge, and permeability, with opportunities to tailor surface chemistry for such requirements such as mucosivity and receptor recognition (as shown for zein/hyaluronic acid/chitosan based complexes) or form a stable egg-box like structure on top of the zein after coating with alginate and drying followed by ionic cross linking of the layer by calcium.

Finally, covalent linking of zein, typically via a controlled Maillard reaction between zein's amino groups and the polysaccharide's reducing end, yields the most robust structure, but the development of sophisticated processes deters its application, as it often creates significant additional regulatory obstacles for food ingredients.

The price for building in more functionality is increased system complexity and consequently, an increasing price to pay in complexity costs: increasing the number of critical process variables to control and process time for each layer added, raising batch to batch reproducibility problem potential, creating higher burden in the regulatory package as every layer must be individually rationalized [1–5]. Thus, the literature points to the simplest strategy first: “binary formulation for the purpose of gastric resistance/aqueous dispensability, ternary and calcium cross-linked formulations to address issues such as gastric resistance or salt tolerance, while multi-layered systems or ligand-modified nanoparticles to be considered if site-specific or programmable delivery is unobtainable by simpler methodology.

Critical Formulation and Process Variables

Comparison across published literature is complicated because a few variables dictate particle properties. The assembly pH dictates the charge of each partner and the magnitude of the electrostatic force; charge density and magnitude also dictate how well the partners interact and how strong the electrostatic repulsive/attractive forces are. The polysaccharide-to-zein mass ratio dictates surface coverage, including where the saturation plateau occurs.

Polysaccharide molecular weight affects shell thickness and loopiness, or the area available

to the mucoadhesive environment; low-molecular-weight ranges produce thinner, denser, more uniform shells, while higher-molecular-weight fractions tend to bridge between particles even at ratios below saturation.

Charge density matters for tolerance to changes in ionic strength, which explains why highly sulfated carrageenan and alginate perform better than low-methoxyl pectin in saline conditions. The amount of zein and ethanol in the feed solution, as well as shear rate and the solvent-to-antisolvent ratio, determine particle core diameter and diameter distribution, and the particle's integrity and residual solvent levels that affect safety are dictated by the method of removal of the remaining ethanol (ultrafiltration, rotary evaporator, or dialysis). Reporting the above-mentioned parameters, as well as the temperature and the ionic composition (e.g., type and concentration of salt) of the assembly solution, is necessary before any lab comparison can be made effectively; the variables and their respective effects on the resultant particles have been summarized above (see Table 2).

Zein/Polysaccharide Systems By Polysaccharide Type

Alginate and Propylene Glycol Alginate

Alginate is a linear anionic polysaccharide derived from brown algae that finds wide applications in the food industry as a thickener, stabilizer, and emulsifier. The standard method of preparing zein/alginate nanoparticles (ZANs) involves pH-induced antisolvent precipitation. ZANs exhibiting a core-shell structure demonstrate excellent colloidal stability at pH 3–8 and in 100 mM NaCl at pH 7. ZANs remain stable at pH 4 in 2 M NaCl and after incubation at 90°C for 120 min at pH 7. ZANs inhibit gastrointestinal degradation of superoxide dismutase (SOD) and amplify the bioactivity of encapsulated limonoids from pomelo seeds.^{7,19,27}

Propylene glycol alginate (PGA), an alginate derivative soluble in aqueous ethanol, is amenable to antisolvent co-precipitation with zein. Zein/PGA nanoparticles (ZPGANs) adopt a characteristic fruit-tree-shaped morphology with zein nanoparticle clusters (‘fruit’) attached to PGA polymer branches (‘branches’). Ca^{2+} ions cross-link this structure, enhancing hydrogen bonding and hydrophobic interactions and significantly

reducing proteolytic digestion by pepsin and pancreatin, thereby resulting in sustained release of resveratrol, quercetin, and α -carotene.^{7,19}

Pectin

Pectin is an anionic structural polysaccharide containing galacturonic acid units. Its gelation characteristics depend on the degree of esterification and Ca^{2+} availability. Pectin carboxyl groups (pK_a 3.5) are fully ionized at pH 4.0, enabling strong electrostatic interaction with positively charged zein nanoparticles (pI 5–6) to produce zein/pectin nanoparticles (ZPNs). Encapsulation of curcumin, resveratrol, tannic acid, and hesperetin in ZPNs enhances aqueous dispersibility, antioxidant and anticancer activity *in vitro*, and bioaccessibility. ZPNs remain stable at pH 2–7 and after thermal treatment (80°C, 1 h, pH 4), but exhibit diminished stability above 50 mM NaCl at pH 4. The exact formulation, particle concentration, storage period, and measurement method would need to be specified for a comprehensive stability assessment. Replacing a fraction of pectin with alginate in ternary formulations enhances stability up to 2000 mM NaCl. The addition of sodium caseinate creates ternary zein/caseinate/pectin nanoparticles that maintain nanoscale size upon redispersal after freeze-drying.^{17,20,28,38}

Chitosan

Chitosan, derived from chitin deacetylation, is a cationic polysaccharide soluble in dilute acids and the only food-grade polysaccharide carrying positive charge at acidic pH. It exhibits mucoadhesive interactions with negatively charged sialic acid residues in intestinal mucus, potentially enhancing oral bioavailability. Zein/chitosan nanoparticles (ZCNs) are prepared via antisolvent precipitation. Chitosan molecular weight substantially influences ZCN properties; effectiveness in release control depends on chitosan molecular weight, charge density, crosslinking, cargo properties, enzyme composition and experimental protocol. Low-molecular-weight chitosan provides superior control over release rates in simulated digestion. Layer-by-layer assembly of zein/hyaluronic acid/chitosan ternary nanoparticles enables controlled release of curcumin and piperine under simulated GIT conditions. Charge inversion (bare zein: +28.9 mV; coated: “34.2 to “41.6 mV) demonstrates successful

anionic shell formation in zein/carboxymethyl chitosan nanoparticles designed to co-encapsulate curcumin and resveratrol.^{29-32,39}

Carrageenan

Carrageenan comprises anionic sulfated polysaccharides extracted from red algae. Iota-carrageenan is most commonly used in the food industry due to its high charge density (pK_a H^+ “2) and gelation characteristics. Zein/carrageenan nanoparticles formed via antisolvent precipitation exhibit significantly better pH stability and storage stability than bare zein nanoparticles. Ca^{2+} connects the anionic outer layer through salt bridges. Assembly involves electrostatic attraction, hydrogen bonding, and hydrophobic interactions. Zein/carrageenan nanoparticles effectively suppress photo- and thermal degradation of co-encapsulated curcumin and piperine and postpone nutrient release under *in vitro* GIT conditions.^{23,33}

Hyaluronic Acid

Hyaluronic acid (HA) is a biocompatible polymer of repeating D-glucuronic acid and N-acetyl-D-glucosamine units with well-established anti-inflammatory and anti-aging properties. HA’s solubility in aqueous ethanol enables antisolvent co-precipitation to produce zein/HA nanoparticles. Electrostatic attraction, hydrogen bonding, and hydrophobic forces drive assembly. HA coating induces zein conformational changes (reduced α -helix content) and enhances physical and thermal stability. Encapsulation of naringenin in zein/HA nanoparticles achieved >80% encapsulation efficiency, sustained release, and enhanced cellular antioxidant activity. Design-of-experiments optimization of ternary zein/casein/HA nanoparticles demonstrated that formulation variables (zein concentration, HA concentration, oil-to-water ratio) critically influence nanoparticle size, polydispersity index, zeta potential, and encapsulation efficiency.^{22,34}

Additional Polysaccharide Systems

Gum Arabic (GA) is a glycoprotein demonstrating broad pH solubility and emulsification activity. Zein/GA nanoparticles remain stable at pH 3–9 but are disrupted by thermal treatment above 70°C. Soybean Polysaccharide (SSP), a negatively charged water-soluble polysaccharide, produces zein/SSP nanoparticles with high encapsulation efficiency and photostability for quercetin. Chondroitin

Sulfate (CS), a linear polysaccharide with repeating galactosamine-glucuronic acid units, forms spherical zein/CS nanoparticles with pH and heat stability, though ionic strength stability is pH-dependent. Ternary zein/CS/sophoro lipid nanoparticles demonstrate superior encapsulation efficiency and anticancer activity against HepG2, MCF-7, and HeLa cells. Other polysaccharides, including HPMC, tea polysaccharides, and agar, have also been employed. Binary, ternary, and layer-by-layer system comparisons are discussed, and circumstances for which additional complexity is useful vs. those for which it works against the system in terms of scale, consistency, or path through regulation.^{21,24-26,35-38}

Stability and Controlled Release

Zein/polysaccharide nanocomplexes achieve colloidal stability primarily through electrostatic repulsion mediated by the polysaccharide shell. At sufficiently high polysaccharide surface density, the magnitude of zeta potential increases, forming an energy barrier that repels particle aggregation across a broad pH range and at moderate ionic strengths. The polysaccharide-to-zein mass ratio represents a critical formulation variable: at low polysaccharide levels, surface coverage remains incomplete; at saturation, excess polysaccharide can promote depletion flocculation.^{5,17-26}

Polysaccharide coating enhances thermal stability through multiple mechanisms: elevated temperature strengthens hydrophobic interactions between zein and shell while suppressing protein-mediated aggregation. However, overheating changes the nanoparticle structure and denatures the thermosensitive payload (e.g. Curcumin), so system optimization depends on the loading.

Ionic strength stability demonstrates strong dependence on polysaccharide charge density: high-charge-density polysaccharides (carrageenan, alginate) tolerate higher salt concentrations than low-charge-density polysaccharides (pectin). Ca²⁺ cross-linking strengthens the shell by forming salt bridges between anionic polysaccharide chains, substantially increasing ionic-strength stability and resistance to enzyme digestion.^{17,23,33}

Encapsulated bioactives exhibit variable release rates in simulated intestinal fluid (SIF) versus simulated gastric fluid (SGF) through multiple mechanisms: These include (i) solubilization by

mixed micelles of bile salts and peptides released during protein digestion, (ii) release of the digestible zein core and subsequent cleavage by pancreatic proteases, (iii) enhanced ionization of the carbohydrate shell at the intestinal pH which results in increased shell hydration and enhanced shell permeability, and (iv) pH mediated structural changes in the tertiary structure of zein resulting in an 'unfolded' core structure. Ca²⁺ cross-linking counters these effects by creating a denser network, yielding more sustained release profiles.^{23,28-34}

Release Kinetics Models

The literature reports employ a variety of release kinetics models (zero-order, first-order, Higuchi, Korsmeyer–Peppas, Weibull, and Peppas–Sahlin) to describe the behavior of the zein/polysaccharide system. The release exponent and goodness-of-fit criteria enable distinction among diffusion, swelling, erosion, and combined mechanisms.

The component breakdown of binding (electrostatic, hydrogen-bonding, and hydrophobic) is determined through thermodynamic analysis of parameters like binding constant, binding number, ΔG , ΔH , and ΔS derived from ITC or variable-temperature binding experiments. Adsorption isotherms apply when polysaccharide adsorption is specifically investigated, with clear statement of assumptions and limitations for heterogeneous biopolymer surfaces.

Bioavailability: Distinctions and Evidence

In vitro release, bioaccessibility, cellular uptake, and in vivo bioavailability are well recognized as distinct events rather than the same concept. No clear relation was expected between in vitro dissolution and in vivo bioavailability. In detail, the following biological data were provided: cell model, dose, exposure time, free-cargo control, blank-carrier control, and cytotoxicity of the unloaded nanoparticles.

Safety Considerations

Comprehensive nanoparticle safety evaluation addresses residual ethanol from manufacturing; zein source, purity, and allergenic potential; polysaccharide molecular weight and endotoxin contamination; long-term storage stability; gastrointestinal fate; and regulatory requirements for food and pharmaceutical applications. Individual ingredients will have important regulatory factors relating to their

identity, purity, proposed use, dosage strength and concentration, formulation, and mode of administration. However, it's worth noting that no composite material formed by the ingredients, by the simple fact of being sold and labelled food grade, would 'automatically' receive approval to be marketed for the purpose intended.

Summary of Key Zein/Polysaccharide Nanoparticle Systems

Future Perspectives and Challenges

Although there has been great progress, several challenges remain before it can be used for wide-scale commercial or clinical application. The goals are to improve the batch-to-batch reproducibility, evaluate the possibility of recovering solvents to minimize environmental impact, scale continuous precipitation via microfluidic and impinging-jet technologies, validate spray-drying and freeze-drying processes for powder production, choose sterilization methods that do not compromise the properties of the nanoparticles, establish shelf-life testing and safety protocols, and implement process analytical technology (PAT) and quality-by-design (QbD) principles.

Scientific Knowledge Gaps

The molecular mechanisms responsible for enhanced bioactivity remain incompletely characterized. Limited *in vivo* pharmacokinetic and biodistribution investigations restrict understanding of real-world efficacy, as most studies remain confined to *in vitro* assessments. Animal and human studies are essential to validate safety, efficacy, and bioavailability before regulatory approval. The article described in the report focused on preparing charge-dependent polysaccharide-zein particles and microscale particle preparation. It also discussed its GI tract release and bioaccessibility, as well as mechanisms of interaction with the help of molecular simulation.

Advanced Design Approaches and Decision Frameworks

Emerging research focuses on multilayered shells with precisely controlled charge density for programmable release and targeted delivery. Surface functionalization with targeting ligands (e.g., lactobionic acid, RGD peptides) offers potential for tissue selectivity. A complete decision-making framework would systematically link the desired application and

content, polysaccharide charge, particle size, release and dissolution system, molecular weight, manufacturing procedure, and formulation design. Increased formulation complexity offers clear advantages in some contexts but can compromise scalability or regulatory acceptance in others.

CONCLUSION

Zein/polysaccharide nanoparticles represent a versatile, sustainable, and food-grade delivery platform that effectively combines the natural encapsulation capability of zein with the stabilizing and functional properties of polysaccharides. These core-shell nanostructures exhibit high encapsulation efficiency, enhanced aqueous dispersibility, improved stability against environmental stresses, and controlled stimulus-responsive release characteristics. The GRAS status of both zein and most polysaccharides, coupled with environmentally friendly fabrication methods that circumvent toxic organic solvents, positions these nanoparticles as promising candidates for applications in functional foods, nutraceuticals, and pharmaceutical delivery systems. Among the available polysaccharides, high-charge-density systems (carrageenan, alginate) offered superior pH stability, calcium-crosslinked systems (PGA, pectin) exhibited excellent ionic strength tolerance, and the pH-responsive and multilayer architectures offered superior controlled release profiles. The remaining barriers to clinical translation include *in vivo* bioavailability, scale-up manufacturability, and lot-to-lot uniformity. The inherently adaptable architecture of zein/polysaccharide systems, including cross-linkable shells and layer-by-layer assembly strategies, offers substantial flexibility to tailor delivery performance to specific applications. Continued advancement in nanoparticle engineering, mechanistic understanding, and large-scale manufacturing are expected to consolidate zein/polysaccharide nanoparticles as a valuable platform for enhancing the stability, bioaccessibility, bioavailability, and therapeutic efficacy of bioactive compounds.

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