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Review article

Chitosan Nanoparticles: Synthesis, Properties, and Drug Delivery

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ABSTRACT

Chitosan nanoparticles (CSNPs) represent one of the most widely researched platforms for allowing drug delivery in pharmaceuticals and they exhibit a high versatility in relation to the numerous uses that they can serve due to their unique qualities as biopolymers. Derived from chitin through deacetylation of that material, chitosan is biocompatible, biodegradable, exhibits mucoadhesive and cationic properties. The tunability of the phospholipid properties of the chitosan molecule (i.e., molecular weight, degree of deacetylation and charge density) makes it possible to design and produce CSNPs that are in the sub-micron to 1 micron size range or less in diameter. This review provides an in-depth coverage of the available literature and empirically investigates the physicochemical basis for the formation of CSNPs based on the following formation techniques: i) ionotropic gelation, ii) emulsification cross-linking, iii) nanoprecipitation, and iv) self-assembly. State-of-the-art characterization techniques, including the use of electron microscopy, dynamic light scattering or other particle sizing techniques, zeta potential, Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), and calorimetry (or thermal analysis), are addressed. Various mechanisms associated with cellular internalization and mucoadhesive/cell-cell adhesion interactions, as well as mechanisms of altering tight junctions and mechanisms by which CSNPs may be released from endosomes, are discussed. Safety assessments available on CSNPs encourage the researcher to consider this agent in the medical application in future projects.

Keywords: Chitosan nanoparticles; Drug delivery; Mucoadhesio, Nanoparticle characterizations

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1. INTRODUCTION

In the last thirty years the rapid progress of the field of nanotechnology has resulted in significant changes in the drug-delivery industry through the development of nanocarriers that are specifically designed to bypass the pharmacokinetic barriers faced by traditional drugs, including: low-solubility in water; fast elimination from the body; nonspecific redistribution within the body; low amounts of drug available for use by the body after administration [1]. Of the many different polymeric nanoparticle systems being studied today, chitosan nanoparticles (CSNPs) have a special role due to the unique ability of chitosan to offer both the advantages of being a naturally derived material (versatile) and its useful biological properties (e.g. mucoadhesive, biodegradable, antimicrobial, and modulatory for immune-cell functions) [2].

Chitosan is a polymer of 2-amino-2-deoxy-D-glucopyranose (poly-β(1→4)-2-amino-2-deoxy-D-glucopyranoside), made from the deacetylation of chitin (the second most abundant natural polymer after cellulose in the world), which is found in the exoskeletons of crustaceans (e.g. shrimp and crab), outer cuticle of insects, and cell walls of fungi. This chemical process produces a semi-crystalline polymer with primary amino (-NH2) groups at the second carbon positions (C-2) of the sugar monomer units. In mildly acidic conditions, the amino groups will carry a positive charge and therefore are distinctly different from most other natural

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polysaccharides (e.g. hyaluronic acid, alginate and heparin) that are negatively charged in pH-neutral or mildly basic conditions. The cationic nature of chitosan provides the basis for the ability of the CSNPs to interact with negatively charged biological surfaces such as mucosal epithelial cells, bacterial membranes, DNA and lipid bilayers in endosomes, and to have a variety of application in promoting the delivery of therapeutics through many different therapeutic avenues (routes) [3,4].

Between 2000 and 2017, the global landscape of research into CSNPs has undergone a dramatic change. The annual number of publications in 2000 was around 50, whereas by 2017 this figure had increased over 800 (according to the Scopus database). The main reason for this growth was due to the convergence of advances in polymer chemistry, nanoparticle fabrication technology, and molecular pharmacology that allowed for the development of more advanced designs of CSNPs. CSNPs were initially developed with relatively simple formulations that involved ionotropically gelling proteins using tripolyphosphate, while subsequent generations were designed with a combination of chemical modifications (e.g., PEGylation, thiolation, and quaternization), surface functionalization using specific ligands (e.g., folic acid, transferrin, and RGD peptides), and inclusion of stimuli-responsive elements responsive to pH, redox potential, enzymes or temperature [5,6]. In the structure of this review, three main sections are included that delineate the journey that a scientist takes from basic polymer science to synthesis of the polymer to characterization, and then finally into numerous biomedical applications with descriptions of mechanistic insights, toxicological evidence, and an overview of potential translation hurdles.

2. CHITOSAN: MOLECULAR ARCHITECTURE, PHYSICOCHEMICAL PROPERTIES, AND BIOLOGICAL ACTIVITY

2.1 Chemical Structure and Degrees of Modification

Chitosan, or chitosan polymer, is a copolymer made up of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc). These two types of molecules are connected to each other by β-(1→4) glycosidic bonds. The most important property that affects how soluble chitosan will be, as well as its ability to bind with other molecules, is the degree of deacetylation (DD). This defines the percentage of GlcN units in chitosan that are deacetylated and therefore, are soluble. The percentage of DD in different chitosan grades ranges from approximately 60% (partially deacetylated) to over 99% (fully deacetylated) with pharmaceutical grades specifying 75-85% DD [7,8]. Chitosan is produced in a variety of molecular weights. The lowest molecular weights (less than 10,000 Da) are typically used for gene delivery and antifungal treatments. The medium molecular weights (50,000 to 310,000 Da) are considered optimal for making nanoparticles and the highest molecular weights (greater than 310,000 Da) are generally used for making films and scaffolds [7,9].

At the solid-state level chitosan is a semi-crystalline material that can be divided into two major types of crystal forms based on how the material was treated. The type I tendon form is made from solely high DD chitosans, while the type II annealed crystal form has been annealed. X-ray powder diffraction can easily identify whether chitosan is in the tendon or annealed state. The amount of crystallinity declines as the DD increases and the polydispersity of the molecular weight as indicated by the effect on the kinetics of dissolution of nanoparticles formed from chitosan [10].

In solution, chitosan behaves as either 1.) an extended random coil (low molecular weight chitosan with a high concentration of salt), or 2.) an elongated worm-like chain (high molecular chitosan with a low concentration of salt), depending on the degree of repulsion between the protonated amino groups due to electrostatic forces. The persistence length of chitosan chains is also affected by both ionic strength and pH and is approximately 7.5 nm [11]. These solution-state conformational properties directly determine nanoparticle nucleation and growth kinetics during synthesis.

2.2 Solubility and pH Behavior

Dilute organic acids (1-2% v/v) at pH under ~6.5 will dissolve chitosan. Examples of these acids include formic, citric, lactic, and acetic acids [12]. When chitosan's amino groups are protonated (pKa 6.3-6.5), they become cationic polyelectrolytes. Chitosan's intrinsic viscosity, indicated by [η], can be described using the Mark-Houwink relationship: $K \cdot M^a$, where K and a are dependent on both the structure of chitosan and the solvent properties. At pH greater than ~6.5, deprotonation occurs and chitosan will precipitate out of solution. This action leads to significant limitations in the use of chitosan in biological systems [13]. Thus, researchers have extensively chemically modified chitosan through methods such as N-trimethylation (to quaternized chitosan, QC, to produce a soluble polymer across all pH), O-carboxymethylation, and N-succinylation. These derivatives of chitosan have retained the biological activities of native chitosan while providing pH-independent solubility for use in parenteral and systemic delivery methods [14].

2.3 Mucoadhesion Mechanisms

Mucoadhesion, the adhesion of a drug delivery system to mucosal surfaces, is a cardinal property of CSNPs that prolongs gastrointestinal residence time, enhances drug absorption at mucosal epithelia, and enables targeted delivery to inflamed mucosa in conditions such as inflammatory bowel disease (IBD). The mucoadhesive mechanism of chitosan is primarily mediated by electrostatic attraction between protonated amino groups ($-NH_3^+$) and the negatively charged sialic acid and sulfate residues of mucin glycoproteins, supplemented by hydrogen bonding and hydrophobic interactions with mucin's hydrophobic domains. The work of mucoadhesion (W_muc) is quantified by tensile and rheological methods, with CSNPs demonstrating significantly higher W_muc values (0.6–1.8 mJ/m²) compared to anionic or non-ionic polymer nanoparticles under equivalent conditions [15, 16].

Beyond simple adhesion, chitosan uniquely disrupts tight junctions (TJs) of intestinal epithelial cells—the primary paracellular permeability barrier by redistributing the scaffolding proteins ZO-1 and ZO-2 and the transmembrane proteins claudin-4 and occludin from their junctional locations. This TJ-opening effect transiently increases transepithelial electrical resistance (TEER) decline and mannitol permeability by 3–8-fold in Caco-2 cell monolayer models, enabling paracellular transport of macromolecular therapeutics (peptides, proteins, oligonucleotides) that are otherwise excluded from this pathway. Critically, this TJ modulation is reversible within 2–4 hours of chitosan removal, confirming a non-cytotoxic mechanism compatible with mucosal safety.Critically, this TJ modulation is reversible within 2–4 hours of chitosan removal, confirming a non-cytotoxic mechanism compatible with mucosal safety [17].

2.4 Intrinsic Biological Activities

Numerous studies have documented the inherent antimicrobial properties of chitosan against different types of microorganisms including viruses, bacteria, and fungi and how this is accomplished by disrupting their membranes [18]. The cationic chains of chitosan electrostatically adsorb to the negatively charged bacterial cell surfaces (lipopolysaccharides in gram-negative bacteria and teichoic acids in gram-positive bacteria), changing the permeability of the cell membranes, causing intracellular leakage of components, and resulting in cell lysis. Under certain conditions with higher concentrations of chitosan, the chitosan can also be taken up into bacterial cells and interfere with nucleic acid and protein synthesis. Minimum inhibitory concentrations (MIC) for chitosan against common pathogens are between 0.1 mg/mL and 2 mg/mL. With the formulation as a nanoparticle, the MIC can be improved up to 5-50 times smaller than without formulation because of increased surface area:volume ratio as well as a more rapid rate of interaction with the bacterial cell membrane [19].

By engaging the pattern recognition receptors expressed on a number of immunologically competent cells (i.e., macrophages, TLR-2, etc.) chitosan can stimulate those cells to enhance defence against pathogens in the form of vaccine adjuvants which can stimulate both humoral and cellular-specific immune responses to vaccines. The clinical application of chitosan has been in the form of wound dressing material (e.g., chitosan HemCon, chitosan ChitoFlex) for controlling haemorrhage in both military and civilian trauma settings and are FDA approved for that purpose [20].

3. SYNTHESIS OF CHITOSAN NANOPARTICLES

Since the initial study on synthetic technologies for CSNP production by Calvo et al. (1997) a wide variety of technologies have been developed and used to manipulate different physical and chemical driving forces to create nanoparticle structures [5]. The selection of the method is extremely important, as it directly affects particle size, surface charge, drug-loading efficiency, stability of the resulting colloid, and the time required to release the drug; these effects are summarized in Table 1.

3.1. Ionotropic Gelation

Ionotropic gelation, the most widely used, simplest, and most scalable method for synthesizing chitosan nanoparticles (CSNP), was also the first to achieve widespread use in pharmaceuticals. This method uses the spontaneous ionic cross-linking of cationic chitosan polymers with multivalent polyanions (usually sodium tripolyphosphate (TPP, $Na_5P_3O_{10}$)) by way of instantaneously forming polyelectrolyte complexes. When an alkaline aqueous chitosan solution (0.1–1 mg/mL, pH 4.5–5.5) is dropped into a solution of TPP (or a reverse), and the two solutions are agitated at room temperature, nanoparticles are formed instantly due to charge neutralization without the need for organic solvents, elevated temperatures, or emulsification [5, 21].

The particle size (size range: 100-800 nm) and Zeta potential (+20 to +40 mV) of nanoparticles prepared using chitosan and tripolyphosphate (TPP) will be a function of multiple factors, including the chitosan:TPP mass ratio (3:1 to 6:1 is optimal), chitosan's molecular weight, chitosan concentration, deacetylation percentage (DD%) and pH of both solution prior to mixing. The three regimes of nanoparticle formation are described by the model of Bhattarai (2004). Regime 1 occurs with TPP concentrations below the critical concentration. At these concentrations positively charged soluble polyelectrolyte complexes form. In regime 2, at intermediate TPP concentrations, very small stable nanoparticles are produced (i.e., the optimal regime) [22]. In regime 3, above the critical TPP concentration, large scale aggregates and gels are produced. Several process parameters have a significant impact on the resulting nanoparticle size distribution including stirring speed (200-1200 RPM), temperature (4-37 °C), ionic strength and addition rate (Jonassen et al., 2012). Nanoparticles prepared using ionotropic gelation and chitosan as the polymer component with a drug that has been dissolved in either the chitosan or TPP phase prior to nano particle formation will have encapsulation efficiencies of between 30-90% for protein molecules and 20-75% for small molecular weight compounds depending upon molecular charge or the extent of hydrophobication [23].

3.2. Emulsification Cross-linking

Chitosan will be emulsified with an oil (i.e., either paraffin, sunflower, or vegetable) in a surfactant-mediated process after being dissolved in acidic aqueous solution, then cross-linked to form a Schiff base cross-linking between the chitosan amine and an aldehyde group using glutaraldehyde or formaldehyde (bonding sequence). The resulting nanodroplet emulsion created from this process can initially set as liquid nanodroplets and be made into solidified nanoparticles through chemical fixation. The resulting nanoparticles can range from approximately 200–1000 nm with a narrow size distribution when either sonication or high-pressure homogenization is used to emulsify [13]. The primary limitations to this technique are the cytotoxicity of the cross-linking agents glutaraldehyde and formaldehyde, thus leading to research on other alternative cross-linking agents such as genipin (a naturally occurring iridoid glycoside), oxalic acid, and epichlorohydrin for potential pharmaceutical applications [24].

Genipin cross-linked chitosan nanoparticles (CSNPs) have been found to be the preferred choice for biomedical uses that require structural integrity through varying pH levels and have the characteristic blue color from the oxygen mediation of polymerization of the genipin-chitosan adduct as well as significantly greater cytocompatibility (IC_{50}: ~10,000-fold more than glutaraldehyde in L929 cells) and slower degradation rates in physiological media. The degree of cross-linking, controllable by the genipin: chitosan ratio and reaction time/temperature, directly modulates drug-release kinetics, from a burst-release profile (low cross-linking) to zero-order sustained release (high cross-linking) [24,25].

3.3. Nanoprecipitation (Solvent Displacement)

Nanoprecipitation is a process that uses the turbulence at the interface of a water-miscible organic solvent containing dissolved chitosan (usually acetone or ethanol) and an aqueous anti-solvent during stirring to create nanoparticles through the rapid diffusion of the organic solvent into the anti-solvent. The resulting supersaturation leads to the collapse of chitosan chains, thereby promoting particle nucleation and the formation of nanoparticles [26]. This technique is well-suited for encapsulating hydrophobic drugs because the drug co-precipitates with chitosan during the solvent-displacement step. Nanoparticles will typically have a final size in the range of 100-400 nm, with particle size influenced by factors such as the injection rate, the organic-to-antisolvent ratio, and the chitosan concentration. One of the major advantages of using this technique is that no high-shear mechanical processing is required, which means that the structural integrity of the fragile biomolecular cargo remains intact [27].

Table 1. Comparison of Chitosan Nanoparticle Synthesis Methods. Table 1. Comparative synthesis parameters for principal chitosan nanoparticle preparation methods. EE, encapsulation efficiency. Data compiled from primary literature 1997–2017.

Method	Size (nm)	Zeta (mV)	EE (%)	Scalability	Best Application
Ionotropic gelation	100–800	+20 to +40	30–90	Very High	Proteins, vaccines, oral delivery
Emulsification/cross-link	200–1000	+15 to +35	40–80	Moderate	Hydrophilic drugs, gene delivery
Nanoprecipitation	100–400	+18 to +38	50–85	High	Hydrophobic drugs, inhalation
Polyelectrolyte complex	100–600	+10 to +30	40–75	High	Oral insulin, gene/siRNA delivery
Self-assembly (amphiphilic)	50–300	+15 to +30	60–95	Moderate	Hydrophobic drugs, cancer therapy
Reverse micellar method	50–200	+20 to +35	55–90	Low	Nucleic acids, fragile macromolecules

3.4. Biophysics of Polyelectrolyte Complexation (PEC)

Electrostatic self-assembly of oppositely charged polyelectrolytes (chitosan and anionic biopolymers) creates PEC nanoparticles (chitosan & biopolymers include hyaluronic acid, alginate, carrageenan, chondroitin sulfate, dextran sulfate, and heparin) without using any chemical cross-linking or organic solvents [28]. The resulting core-shell or interpenetrating network structure exhibits stimulus-response behaviour. For example, the anionic component protonates/alloys together as they approach/are in the acidic environment of the stomach (1.2-3pH) resulting in the disintegration of the PEC and the potential for early drug release from the PEC nanoparticles. Conversely, as digestion processes take place and the ions in the anionic component of the PEC become fully ionized in the alkaline conditions of the intestines (6.8-7.4pH), the complex remains stable, resulting in delayed drug release from the PEC nanoparticles. Chitosan/alginate PEC nanoparticles have also been explored as an option for oral delivery of insulin by taking advantage of the complementary attributes of these polymers - i.e. the cationic nature of chitosan that opens tight junctions, while the pH responsive swelling of alginate helps to maintain a tightly bound complex with the gastrointestinal tract [29].

3.5. Self-Assembly of Hydrophobically Modified Chitosan

Amphiphilic chitosan derivatives have been formed by partial substitution with hydrophobic moieties (palm). These derivatives have formed core-shell nanostructures in aqueous solution via hydrophobic self-assembly similar to block copolymer micelles. The poor aqueous solubility of many drugs makes them susceptible to inclusion into the hydrophobic core of the chitosan derivatives, while the hydrophilic outer layer imparts colloidal stability and functionality to the drug-loaded core. CMCs for hydrophobically modified chitosans are generally in the range of 0.01–0.2 mg/mL, and aggregation number and core size are dependent on the degree of substitution and the length of the hydrophobic chain. Additionally, the nanostructures exhibit a high degree of drug loading (60–95%) for hydrophobic drugs and inherent stimuli responsiveness due to the ionizable chitosan backbone [30, 31].

4. PHYSICOCHEMICAL FEATURE OF CSNP

4.1. Size, Morphology, and Internal Architecture

The first size of CSNPs can be determined by the use of Transmission Electron Microscopy (TEM) in a negative staining technique (e.g., uranyl acetate, phosphotungstic acid), which allows for direct measurement and visualization of the cores' diameter (typically 50-500 nm) using ionotropic gelation [32]. High-resolution transmission electron microscopy (HRTEM) is able to distinguish the difference between amorphous (majority of CSNPs) and semicrystalline particle types, showing chitosan's degree of crosslinking as a function of electron density within the particle. Atomic Force Microscopy (AFM) in a Tapping Mode will measure surface morphology and mechanical properties (Young's Modulus through nanoindentation) of the CSNPs while in their native hydrated state and provide complementing morphological data to that established using TEM, without any dehydration artifact. Nanoparticle Tracking Analysis (NTA) using a NanoSight instrument combines light scattering and fluorescence microscopy to provide a size distribution and number density (concentration) of individual CSNPs within suspension; the NTA technique is particularly sensitive to size variation in heterogenous (polydisperse) particle populations where dynamic light scattering (DLS) can misrepresent average sizes due to DLS's intensity-averaged (z-average) algorithm [33].

Through dynamic light scattering (DLS), it is found that the hydrodynamic diameter shows a consistent difference of 20-100 nm when compared to the core diameter as measured by transmission electron microscopy (TEM) because of an associated polymer layer and any hydrated surface molecules. DLS uses the cumulant fitting algorithm to generate z-average diameter and polydispersity index (PDI). A PDI < 0.20 indicates there is an appropriate monodisperse population for pharmaceutical use. Using multi-angle DLS (MADLS) and DLS particle size distribution (PSD) algorithms provides the most accurate particle size distribution analysis for polydisperse CSNP samples. The PDI of a sample is an important quality metric that needs to stay constant for its entire shelf-life; increases in PDI > 0.05 units indicate an unstable colloidal suspension which can trigger molecular aggregation before ending the shelf life of a product [33].

4.2. Surface Charge and Colloidal Stability

Laser Doppler Electrophoresis is a technique commonly used to report the zeta potential (ζ) of whole nanoparticle colloids (CSNPs) that have been dispersed in dilute solutions. The zeta potential of freshly prepared CSNPs in buffered acetate solution (pH = 4.5 5.5) typically ranges for zeta potentials of +20 to +45mV. This positive zeta potential reflects the protonated surface of chitosan. At physiological pH, the zeta potential falls to +5 to +15mV due to less protonation of amino groups on chitosan, and additional agents used to provide stabilizing influences on CSNPs dispersed in physiological media typically include steric stabilization provided by polyethylene glycol and the use of poloxamer surfactants via adsorption. The kinetics of aggregation of CSNPs in simulated biological fluids (including simulated gastric fluid, simulated intestinal fluid, phosphate-buffered saline and serum-free medium containing 10% (v/v) foetal bovine serum) are measured over time using time-resolved dynamic light scattering with effective aggregation being defined as being capable of remaining stable for >72 hours (for use in vivo) after dispersion in those media [34, 35].

When nanoparticles come into contact with a fluid, a layer of proteins from the fluid will stick to them, which is referred to as the protein corona. This layer of proteins can then greatly affect how the nanoparticles behave in terms of their ability to be taken up by cells, their ability to be distributed throughout an organism, and their properties as a colloid. The proteins that are part of the protein corona can include: albumin, fibrinogen, IgG (immunoglobulin G), and apolipoproteins; these are the main proteins that were identified in studies using mass spectrometry and gel electrophoresis (SDS-PAGE). The composition of the protein corona will depend on both the properties of the nanoparticles as well as the composition of the fluid. In general, when nanoparticles become coated with protein, their surface zeta potential will change from a value of +30 mV to +5 mV and their hydrodynamic diameter will increase by 30 to 80 nm; therefore, for preclinical studies, characterization of nanoparticles should be done in a relevant biological medium rather than simply in a buffer [36].

4.3. FTIR and Raman Spectroscopic Characterization

ATR-FTIR provides a definitive means to confirm the chemical structure of chitosan within nanoparticles and to establish that both drug encapsulation and cross-linking chemistry have occurred. The FTIR bands for chitosan include the following: O-H/N-H stretch (3450 cm^{-1}); C-H stretch (2920 cm^{-1}); Amide I (C=O stretch, 1655 cm^{-1}); Amide II (N-H bend, 1590 cm^{-1}); C-N stretch (1380 cm^{-1}); Asymmetric C-O-C stretch (1155 cm^{-1}); and C-O stretch (1076 cm^{-1}). When chitosan is cross-linked with TPP, the N-H bend occurs at 1530–1545 cm^{-1}. There are also stretching vibrations of P=O (1220 cm^{-1}), P-O-C (1076 cm^{-1}) and P-O (890 cm^{-1}) when cross-linked with TPP. When chitosan is cross-linked with Genipin, an imine (C=N) band appears at 1640 cm^{-1}, causing the amide II band to shift. ^{1}H and ^{13}C NMR spectroscopy of the dissolved samples will allow accurate determination of DD (±1%) and confirm drug loading by identifying drug-specific resonances [37, 38].

4.4. Drug Release Profiling

When evaluating in vitro release of drug from CSNP, it is recommended to use physiologic buffer solutions with the use of either the dialysis membrane diffusion method, which is the most widely used, or resuspension-centrifugation. Both methods are performed at 37 degrees Celsius with sink conditions maintained during the entire experiment. Three phases are used to characterize release profiles: a fast initial burst release (1-6 hours) due to either poorly encapsulated drug or surface associated drug; followed by a plateau phase that is controlled by diffusion of the drug; and finally a terminal release phase that is dependent upon polymeric erosion [39, 40]. Computer models are developed for each individual release profile and were classified using the zero-order, first-order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell equations. If the Korsmeyer-Peppas exponent is less than 0.45, it can be determined that the transport mechanism is pure Fickian diffusion; if the exponent is between 0.45 and 0.89 it is determined that the mechanism of transport is anomalous (a combination of diffusion and erosion); and if the exponent is approximately equal to 0.89, it is determined that the transport mechanism is Case II (polymer relaxation-controlled).

5. CONCLUSIONS

The current nanoparticles of CSNPs represent a key advance in drug delivery, valued for their biodegradability, biocompatibility, mucoadhesion, and cationic charge. These properties allow them to overcome longstanding challenges in traditional drug delivery, such as enzymatic degradation in the gastrointestinal tract, poor mucosal permeability, low drug solubility, non-specific drug distribution, chemotherapy resistance, and nuclease vulnerability. In the cells, CSNPs provide mechanistic insights, including mucoadhesion, tight junction modulation, and endosomal escape, that can guide next-generation formulation design. Their safety is supported by in vivo toxicology, genotoxicity, and hemocompatibility data, collectively establishing a favorable therapeutic window for clinical translation.

Table 2. Comprehensive Characterization Parameters for Chitosan Nanoparticles using different methods.

Technique	Parameter Measured	Typical Values / Outcome	Regulatory Relevance
TEM / AFM	Core size, morphology	50–500 nm, spherical	FDA guidance: mandatory
DLS (z-average, PDI)	Hydrodynamic diameter	100–600 nm, PDI < 0.25	ICH Q8: critical CQA
Zeta potential (LDE)	Surface charge (mV)	+20 to +45 mV (pH 5)	Stability predictor
ATR-FTIR / NMR	Functional group identity	N-H, C-N, cross-link bonds	Chemical identity/purity
PXRD / DSC	Crystallinity, thermal properties	Semi-crystalline → amorphous	Polymorphism control
Encapsulation efficiency	Drug loading, EE%	30–95%, drug-specific	Dose accuracy
In vitro drug release	Release kinetics, model fit	Korsmeyer-Peppas, n value	Bioequivalence surrogate
BET / BJH	Surface area (m^2/g)	5–50 m^2/g	Batch consistency

The integration of CSNPs with emerging technologies of drug delivery, cell membrane shielding, theranostics, and microfluidic manufacturing positions them as a versatile platform for tackling complex medical challenges.

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Conflict of interest

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Ethical Approval

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CRediT authorship contribution statement

Saleh GM. Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

All authors have read and agreed to the published version.

Availability of data and materials

All information used is available in the cited **literature**.

6. REFERENCES

[1] **Din FU, Aman W, Ullah I, Qureshi OS, Mustapha O, et al.** (2017) Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *Int J Nanomedicine* **12**:7291-7309. doi: 10.2147/IJN.S146315. PMID: 29042776; PMCID: PMC5634382.

[2] **Ahmed TA, Aljaeid BM**. (2016) Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery. *Drug Des Devel Ther* 10:483-507. doi: 10.2147/DDDT.S99651. PMID: 26869768; PMCID: PMC4734734.

[3] **Prego C, García M, Torres D, Alonso MJ.** (2005) Transmucosal macromolecular drug delivery. *J Control Release* **101**(1-3):151-62. doi: 10.1016/j.jconrel.2004.07.030. PMID: 15588901.

[4] **Landriscina A, Rosen J, Friedman AJ.** (2015) Biodegradable chitosan nanoparticles in drug delivery for infectious disease. *Nanomedicine* (*Lond*) **10**(10):1609-19. doi: 10.2217/nnm.15.7. PMID: 26008195.

[5] **Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ.** (1997) Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. *J Appl Polym Sci* **63**(1), 125-132.

[6] **Makhlof A, Tozuka Y, Takeuchi H.** (2011) Design and evaluation of novel pH-sensitive chitosan nanoparticles for oral insulin delivery. *Eur J Pharm Sci* **42**(5):445-51. doi: 10.1016/j.ejps.2010.12.007. PMID: 21182939.

[7] **Younes I, Rinaudo M.** (2015) Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Mar Drugs* **13**(3):1133-74. doi: 10.3390/md13031133. PMID: 25738328; PMCID: PMC4377977.

[8] **Dash, M., Chiellini, F., Ottenbrite, R. M., & Chiellini, E.** (2011). Chitosan—A versatile semi-synthetic polymer in biomedical applications. *Prog Polym Sci 36*(8): 981-1014. DOI: 10.1016/j.progpolymsci.2011.02.001

[9] **Huang M, Khor E, Lim LY.** (2004) Uptake and cytotoxicity of chitosan molecules and nanoparticles: effects of molecular weight and degree of deacetylation. ***Pharm Res*** *21*(2): 344-353. DOI: 10.1023/b:pham.0000016249.52831.a5.

[10] **Kumirska J, Czerwicka M, Kaczyński Z, Bychowska A, Brzozowski K, et al.** (2010) Application of spectroscopic methods for structural analysis of chitin and chitosan. *Mar Drugs* **8**(5):1567-636. doi: 10.3390/md8051567. PMID: 20559489; PMCID: PMC2885081.

[11] Schatz C, Viton C, Delair T, Pichot C, Domard A. (2003) Typical physicochemical behaviors of chitosan in aqueous solution. *Biomacromolecules*, *4*(3), 641-648. DOI: 10.1021/bm025724c.

[12] **Pillai CKS, Paul W, Sharma CP (2009)** Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Prog Polym Sci* ***34***(7): 641-678.

[13] **Agnihotri SA, Mallikarjuna NN, Aminabhavi TM.** (2004) Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J Control Release* 100(1):5-28.

[14] **Kean T, Thanou M. (**2010) Biodegradation, biodistribution and toxicity of chitosan. *Adv Drug Deliv Rev 62*(1): 3-11.

[15] **Sogias IA, Williams AC, Khutoryanskiy VV.** (2008) Why is chitosan mucoadhesive? Biomacromolecules **9**(7): 1837–1842.

[16] **Ramya R, Venkatesan J, Kim SK, Sudha PN.** (2012) Biomedical applications of chitosan: an overview. *J Biomater Tissue Eng* **2**(2):100-111.17.

[17] **Kumar A, Vimal A, Kumar A.** (2016). Why Chitosan? From properties to perspective of mucosal drug delivery. *Int J Biol Macromol* **91***:* 615-622.

[18] **Goy RC, Britto DD, Assis OB.** (2009) A review of the antimicrobial activity of chitosan. *Polímeros* **19**:241-247.

[19] **Kong M, Chen XG, Xing K, Park HJ.** (2010) Antimicrobial properties of chitosan and mode of action: a state of the art review. *Int J Food Microbiol* **144**(1):51-63.

[20] **Muzzarelli RA**. (2010) Chitins and chitosans as immunoadjuvants and non-allergenic drug carriers. *Mar Drugs* **8**(2):292-312.

[21] **Rajitha P, Gopinath D, Biswas R, Sabitha M, Jayakumar R.** (2016) Chitosan nanoparticles in drug therapy of infectious and inflammatory diseases. *Expert Opin. Drug Deliv 13*(8): 1177-1194.

[22] **Gan Q, Wang T, Cochrane C, McCarron P.** (2005) Modulation of surface charge, particle size and morphological properties of chitosan–TPP nanoparticles intended for gene delivery. *Colloids and Surfaces B: Biointerfaces 44*(2-3): 65-73.

[23] **Jonassen H, Kjøniksen AL, Hiorth M.** (2012) Stability of chitosan nanoparticles cross-linked with tripolyphosphate. *Biomacromolecules*, **13**(11): 3747-3756.

[24] **Muzzarelli RAA.** (2009) Genipin-crosslinked chitosan hydrogels as biomedical and pharmaceutical aids. *Carbohydr Polym* **77**(1):1-9.

[25] **Li Q, Wang X, Lou X, Yuan H, Tu H,** et al. (2015) Genipin-crosslinked electrospun chitosan nanofibers: Determination of crosslinking conditions and evaluation of cytocompatibility. *Carbohydr Polym* **130**:166-74.

[26] **Li F, Chen Y, Liu S, Qi J, Wang W, et al. (**2017) Size-controlled fabrication of zein nano/microparticles by modified anti-solvent precipitation with/without sodium caseinate. *Int J Nanomedicine* **12**:8197-8209.

[27] **Mora-Huertas, C. E., Fessi, H., & Elaissari, A. (2010)** Polymer-based nanocapsules for drug delivery. *Int J Pharm* **385**(1-2): 113-142.

[28] **Conzatti G, Faucon D, Castel M, Ayadi F, Cavalie S, Tourrette A.** (2017) Alginate/chitosan polyelectrolyte complexes: A comparative study of the influence of the drying step on physicochemical properties. Carbohydr Polym **172**:142-151.

[29] **Nur M, Vasiljevic T.** (2017) Can natural polymers assist in delivering insulin orally? *Int J Biol Macromol* **103**:889-901.

[30] **Bendjeriou-Sedjerari A, Azzi JM, Abou-Hamad E, Anjum DH, Pasha FA,** et al. (2013) Bipodal surface organometallic complexes with surface N-donor ligands and application to the catalytic cleavage of C-H and C-C bonds in n-butane. *J Am Chem Soc* **135**(47):17943-51.

[31] **Hua D, Jiang J, Kuang L, Jiang J, Zheng W, Liang H.** (2011) Smart chitosan-based stimuli-responsive nanocarriers for the controlled delivery of hydrophobic pharmaceuticals. *Macromolecules* **44**(6):1298-1302.

[32] **Desai KG.** (2016) Chitosan Nanoparticles Prepared by Ionotropic Gelation: An Overview of Recent Advances. *Crit Rev Ther Drug Carrier Syst* **33**(2):107-58.

[33] **Rigaux G, Gheran CV, Callewaert M, Cadiou C, Voicu SN, et al.** (2017) Characterization of Gd loaded chitosan-TPP nanohydrogels by a multi-technique approach combining dynamic light scattering (DLS), asymetrical flow-field-flow-fractionation (AF4) and atomic force microscopy (AFM) and design of positive contrast agents for molecular resonance imaging (MRI). *Nanotechnol* **28**(5):055705.

[34] **Casettari L, Vllasaliu D, Castagnino E, Stolnik S, Howdle S, et al.** (2012) PEGylated chitosan derivatives synthesis and applications. *Prog Polym Sci* **37**(5):659-685.

[35] **He C, Hu Y, Yin L, Tang C, Yin C.** (2010) Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. *Biomaterials* **31**(13):3657-66.

[36] **Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA.** (2008) Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proc Natl Acad Sci U S A* **105**(38):14265-70.

[37] **Bhumkar DR, Pokharkar VB.** (2006) Studies on effect of pH on cross-linking of chitosan with sodium tripolyphosphate: a technical note. *AAPS PharmSciTech* **7**(2):E50.

[38] **Butler MF, Ng YF, Pudney PD.** (2003) Mechanism and kinetics of the crosslinking reaction between biopolymers containing primary amine groups and genipin. *J Polym Sci Part A: Polym Chemistry* **41**(24):3941-3953.

[39] **D'Souza SS, DeLuca PP.** (2006) Methods to assess in vitro drug release from injectable polymeric particulate systems. *Pharm Res* **23**(3):460-74.

[40] **Bragagni M, Beneitez C, Martín C, Hernán Pérez de la Ossa D, Mura PA, Gil-Alegre ME.** (2013) Selection of PLA polymers for the development of injectable prilocaine controlled release microparticles: usefulness of thermal analysis. *Int J Pharm* **441**(1-2):468-75.

[41] **Buwalda SJ, Boere KW, Dijkstra PJ, Feijen J, Vermonden T, Hennink WE.** (2014) Hydrogels in a historical perspective: from simple networks to smart materials. *J Control Release* **190**:254-73.

[42] **Siepmann J, Peppas NA.** (2001) Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Adv Drug Deliv Rev* **48**(2-3):139-57.

[43] **Verma A, Dubey J, Verma N, Nayak AK.** (2017) Chitosan-Hydroxypropyl Methylcellulose Matrices as Carriers for Hydrodynamically Balanced Capsules of Moxifloxacin HCl. *Curr Drug Deliv* **14**(1):83-90.

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