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# Chitosan Nanoparticles in Oral Drug Delivery: A Comprehensive and Equivocal Review

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## Abstract

Oral medications are prevalent within modern-day society because of their convenience for patients and their cost-effectiveness. However, challenges such as poor solubility and gastrointestinal degradation decrease their effectiveness. Recently, advancements in nanotechnology have improved the efficiency of drug delivery, with specialized nanocarriers being developed and synthesized for different functions. This review is concentrated on Chitosan Nanoparticles (CSNPs), which are derived from chitosan, a polysaccharide that is biodegradable and offers a promising solution for overcoming certain challenges associated with drug delivery. CSNPs have been synthesized using a variety of methods such as emulsion cross-linking, ionic gelation and biosynthesis and more novel methods such as incorporation of a magnetic core. These methods have shown improvements in particle stability, CSNP solubility and targeted drug delivery, however, challenges like aggregation remain and must be addressed to optimize the performance of CSNPs. Overall, CSNPs offer a promising advancement in the usage of nanoparticles in medicine with important implications for improving oral drug delivery systems and creating innovative solutions within healthcare.

**Keywords:** Chitosan Nanoparticles (CSNPs); Nanotechnology; Targeted drug delivery; Synthesis methods; Biodegradable polysaccharides

**Abbreviations:** CSNPs: Chitosan Nanoparticles; TPP: Tripolyphosphate; CMCSNPs: Carboxymethyl Chitosan Nanoparticles; QCSNPs: Quaternized Chitosan Nanoparticles; TGA: Thioglycolic Acid; NPs: Nanoparticles

## Introduction

Oral medications are the most common due to convenience, patient preference, cost and easy manufacturing. Oral medications take up 60% of the market for commercial drugs. Furthermore, 84% of top-selling brand drugs are oral. With a market valued at just over $35 billion with 10% annual growth, Oral medications have an exorbitant market share [1,2]. Though oral drug delivery is convenient, it still has challenges, such as poor solubility, due to which only a minimal amount of the drug reaches the bloodstream [3,4]. These poorly soluble drugs also have difficulty in dissolving and permeating in the gastrointestinal tract [5]. In addition, these drugs can be destroyed by the acidic environment present in the stomach and intestines, hence resulting in fewer active drugs being left in circulation [6].

In recent years, advances stemming from the field of nanotechnology and nanomedicine have contributed to the development and assimilation of nanocarrier systems for the oral delivery of drugs. Nanomedicine is particulate matter developed at a nanoscale (1 to 1000nm). Some of these nanometer-sized particles have a propensity to act as carriers in different modes of pharmaceutical delivery systems [7]. After injection by the intravenous route, these nanoparticles have the ability to remain in circulation for long periods of time with a slight reduction of the renal clearance compartment because of their small physical

size and high drug loading and encapsulation efficiency [8]. They allow for sustained or controlled release of drugs, which increases bioavailability and lowers the number of doses. This technique can also solve the problem of drug resistance because it enables cells to internalize more of the drug [9]. In addition, nanoparticles can be designed to contain targeting ligands, which increases the delivery of drugs to desired sites and reduces the amount of drugs delivered to normal tissues [10].

Various polymers, spanning natural, synthetic and semisynthetic categories, are used to create nanoparticles for drug delivery. Among these, chitosan and its derivatives stand out, particularly for oral administration. The most important derivative of chitin is its chitosan, the latter appearing when the acetate group is removed from chitin. The distinct character of chitosan, in particular its cationic charge owing to amino groups, helps drugs to easily cross the barrier and shows great adhesion to mucosal tissues. Presently, it is also the most common mucoadhesive polymer used for oral delivery of proteins and peptides. Moreover, chitosan possesses other attractive characteristics, including being biodegradable, biocompatible and low in toxicity, which makes it ideal for use in pharmaceuticals [11]. Chitosan is a polysaccharide consisting of N-acetyl-d-glucosamine and d-glucosamine linked by 1-4-β-glycosidic bonds and is produced through the deacetylation of chitin in an alkaline medium [12,13]. Chitosan Nanoparticles (CSNPs) are the most advantageous in cases of ocular as well as oral drug delivery systems. Chitosan can be obtained from the fungal cell wall and prawn and crab shells [14]. In particular, transformed chitin consists of chitosan obtained as a result of the deacetylation of purified chitin. The degree of deacetylation can be adjusted using variables such as concentration, chitin to alkali ratios, temperature, the chitin source and reaction extent etc. to control the final product properties, such as molecular weight and a desired pKa (6-7.5) [15,16].

CSNPs have proven to successfully solve challenges in the current insulin delivery method, which is infamous for frequent injections and reduced patient compliance. Preparation methods like coacervation and spray drying are used to create these CSNP carriers, which have shown promise in preclinical studies as oral insulin carriers despite the need for further improvements. Modifying chitosan through techniques like thiolation and grafting can enhance its solubility, permeability and insulin protection in the gastrointestinal tract. Various chitosan carriers such as nanoparticles, microparticles and beads have shown potential in improving insulin absorption in the colon [17].

This review aims to examine the synthesis methods, key properties and bioavailability-enhancing mechanisms of chitosan nanoparticles, with a particular focus on their mucoadhesive capabilities and protective roles in the gastrointestinal tract and how they are currently advancing in the healthcare and nanotechnology market.

## Chitosan Nanoparticles: Synthesis and Properties

Chitosan nanoparticles can now be synthesized using a variety of methods, each with its own benefits depending on the desired application. The first recorded synthesis of chitosan nanoparticles was completed in 1974 by Ohya et al. [18] for use in chemotherapy. They utilized glutaraldehyde as a cross-linking agent to stabilize the nanoparticles [18]. Ohya et al. [18] used a technique known as emulsion cross-linking, where the chitosan is dispersed into an oil phase and linked using a water-soluble cross-linker. This method was also successfully used by Yanat et al. [19] to prepare chitosan NPs that could be used in biodegradable packaging [19]. Emulsion cross-linking allows for specific particle sizes with certain surface properties to be obtained. This method represents a significant milestone in the development of nanoparticle synthesis methods and sets the foundation for research on applying these nanoparticles to drug delivery situations.

Ionic gelation is another method that is commonly used for synthesizing chitosan nanoparticles, in which there are ionic interactions between chitosan and anionic cross-linkers, including Tripolyphosphate (TPP). Due to the amino groups present in chitosan, it has a positive charge, which will interact with the negatively charged ions from the cross-linker, creating NPs. Without the need to use organic solvents, this method is valued for its environmental friendliness and simple methodology.

Recently, studies that have used ionic gelation have focused on the optimization of the synthesis process to improve certain aspects of the NPs. Kharmohammadi et al. [20] showed that using TPP as a cross-linker enhances the percentage yield and the drug loading capacity of the NPs compared to a more traditional cross-linker [20]. They found that TPP improves the stability and uniformity of the NPs while simultaneously increasing the yield, making this method viable for a variety of applications. The improvements attributed to using TPP in ionic gelation are due to its ability to form stronger interactions with the chitosan, leading to the enhancement of the NPs. Hoang et al. [21] also support the idea that TPP-based ionic gelation can achieve highly regulated particle sizes, which is an essential component of targeted drug delivery, especially for poorly soluble drugs [21].

Other than ionic gelation, various methods, such as biosynthesis, are constantly being investigated for increased efficiency and reduced energy consumption. For instance, research [22] conducted by El-Naggar [22] in 2022 biosynthesized chitosan nanoparticles using an aqueous extract of Eucalyptus globulus Labill fresh leaves as a bio-reductant. The maximum yield of chitosan nanoparticles in this process was 9.91mg/mL at pH of 4.5, chitosan concentration of 1%, incubation time of 60 min and temperature of 50 °C. The crystallinity, particle size and morphology of the biosynthesized chitosan nanoparticles were characterized. The chitosan nanoparticles possess a positively charged surface of 31.1mV. Moreover, these biosynthesized chitosan nanoparticles are thermally stable, confirming their durability and stability. These biosynthesis of the chitosan nanoparticles hints at a greener application of the chitosan in the future as this method is simple and requires low energy input.

Chitosan is widely regarded for its strong mucoadhesive nature, as it typically allows for the NPs to attach to linings with mucus

throughout the body, further increasing the time. However, this is not without challenges, as according to Ways M et al. [12] chitosan's derivatives have limited water solubility at neutral and basic pH levels. Therefore, there will not be an issue for nanoparticles that are delivering drugs in acidic environments, such as the stomach, but for organs like the intestines, this will create difficulties in drug delivery.

One method of improving the water solubility, outlined by Farag et al. [23], is carboxymethylation of chitosan. Carboxymethylation introduces carboxymethyl groups (-$CH_2$-COOH) into the structure of chitosan. The carboxymethyl groups improve the solubility because the groups are hydrophilic (water-attracting), meaning that the chitosan has a higher affinity for water, resulting in a higher solubility and better dispersion of the nanoparticles in stable environments. The newly enhanced carboxymethyl chitosan can be used in a variety of biomedical situations, especially drug delivery, with a better outcome than normal CSNPs [24].

These Carboxymethyl Chitosan Nanoparticles (CMCSNPs) were synthesized using the method indicated by Farag et al., where the carboxymethyl chitosan was combined with polyvinyl alcohol, forming nanogels. The nanogels formed with this procedure range in size from only a few nanometers to 1,000 nanometers and have a large surface area with good surface properties. Thus, with more optimal properties, the release of drugs can be better controlled with greater stability. With such a minuscule size, the nanogels can cross biological boundaries including cell membranes, to reach specific sites within cells, ultimately making the CMCSNPs more applicable to situations where a controlled release of drugs is necessary. Furthermore, the better release properties of CMCSNPs result in more effective performance in environments with varying pH levels throughout the body, highlighting their implications in the biomedical field.

In addition to the carboxymethylation of the CSNPs, other adjustments can be made to make the NPs better suited for specific scenarios. For example, the study conducted by Farag et al. [23] in 2014 outlined the novel synthesis of chitosan nanoparticles with magnetic cores. This was done by coating iron (II, III) oxide, also known as magnetite ($Fe_3O_4$) with the chitosan to allow for specific sites to be targeted for delivery of drugs utilizing magnetic fields to move the NPs.

However, one of the limitations of using this method is that the magnetic CSNPs typically aggregate, reducing the potential for dispersibility throughout a broader region. Therefore, their effectiveness in reaching certain parts of the body is limited. The Transmission Electron Microscopy (TEM) images within the study show that the nanoparticles showed signs of aggregation, reducing uniform distribution within the solution. This is a challenge in situations where even dispersal of the nanoparticles is essential for effectiveness of the drug treatments. This method was successful in synthesizing magnetic CSNPs, however, further refinement of the synthesis process or the use of stabilizing agents to prevent aggregation is necessary.

Quaternization of chitosan is a significant modification that can improve the functionality of CSNPs by adding quaternary ammonium groups into the molecule of chitosan [25]. This can be achieved by reacting quaternizing agents, which can take the form of alkyl halides, with chitosan. Specifically, the quaternizing agent glycidyl trimethylammonium chloride has been used by Cai et al. [26] to successfully prepare Quaternized Chitosan Nanoparticles (QCNPs). These QCSNPs also exhibited better solubility, similar to CMCSNPs and can be used in a variety of biomedical applications, especially in wound healing. This is a direct result of the NPs synthesized by Cai et al. having a permanent positive charge, improving their ability to interact with negatively charged cell membranes. Also, the positive charge results in improved adhesion properties, making QCNPs especially useful in promoting tissue regeneration.

The study conducted by Jia et al. expands on the information presented by Cai et al. [26], further emphasizing the use of QCNPs for antibacterial applications [27]. As previously mentioned, the positive charge on the quaternary ammonium salt of chitosan results in bacterial cell death when interacting with the negatively charged cell walls of bacteria. The quaternized ammonium salts of chitosan were tested on *E. coli* bacteria and higher degrees of substitution within the chitosan molecules resulted in more evident antibacterial effects. Therefore, the properties of QCSNPs allow for the NPs to be used as carriers for drugs for specifically targeted cancerous or infected cells [28]. Since the NPs would have a stronger bond to the negatively charged cell walls, this could prolong the residency time within the problematic cells while simultaneously reducing the impact on healthy cells.

Thiolation is another method to increase the mucoadhesion effect-especially the permeability of the nanoparticles-of chitosan nanoparticles. Thiolation is a process in which thiols - R−SH - are introduced into the chitosan by directly reacting any chemical compounds with the thiol group with chitosan. This technique [29] has been pioneered by Bernkop-Schnürch et al. [29] to enhance the mucoadhesion of polymers for pharmaceutical and biomedical applications. The derivatives of the thiolation include cysteine, Thioglycolic Acid (TGA), 2-iminothiolane or 4-thiobutylamidine, N-acetyl cysteine, isopropyl-S-acetylthioacetimidate and glutathione. According to the research conducted by Ways et al. chitosan tablets showed superior cohesion over the chitosan tablets which could be due to the formation of intra/intermolecular disulfide bonds as a result of the oxidation of the thiol groups in thiolated chitosan. This improved cohesion is desirable for the mucoadhesion and the design of controlled-release dosage forms.

The mucoadhesive properties of chitosan, if not modified, pose a huge advantage as the time to permeate and react increases, but also a threat, as these chemicals, if not biodegraded, might cause several issues, such as so promptly into the body. However, according to research conducted by Kean et al. [30] chitosan is thought to be degraded in vertebrates predominantly by lysozyme and bacterial enzymes in the colon. According to Onishi et al. [31] in 2009, lysozyme, in particular, has been found to degrade chitosan

efficiently; 50% acetylated chitosan had 66% loss in viscosity after a 4 h incubation in vitro at pH 5.5 (0.1 M phosphate buffer, 0.2M NaCl, 37 °C). This degradation appeared to be dependent on the degree of acetylation, with degradation of acetylated chitosan (more chitin-like) showing a faster rate. In general, chitinases in microorganisms hydrolyze N-acetyl-β-1,4-glucosaminide linkages randomly, i.e., endo-chitinases (EC 3.2.1.14). Moreover, both the rate and extent of chitosan biodegradability in living organisms are dependent on the Degree of Deacetylation (DD). According to Yang et al. [32] research in 2007, increasing DD would decrease the degradation rate. The extent of degradation is related to the rate, as all the studies are conducted over a finite lifetime. Thus, from the results of these studies, it is likely that, given adequate time and appropriate conditions, the chitosans would degrade sufficiently for consequent excretion.

Another factor to consider when studying the effects of chitosan is biodistribution so that the exact extent to which the medications encased by the chitosan will be distributed within the body can be determined. The oral dosage forms use chitosan as an excipient, although chitosan does not strictly fit the definition of excipient as it has many biological effects. It has been suggested that chitosan chelates fat and reduces cholesterol, but this and its mechanism are somewhat debatable. Apart from the impact that chitosan may have on bile salts and gastrointestinal milieu, the uptake of chitosan into the bloodstream is generally not investigated in oral administration studies.

For the safety and toxicity of the chitosan nanoparticles, chitosan is widely regarded as being a non-toxic, biologically compatible polymer [33]. It is approved for dietary applications in Japan, Italy and Finland [34] and it has been approved by the FDA for use in wound dressings [35]. The modifications made to chitosan could make it more or less toxic and any residual reactants should be carefully removed. However, the exact toxicity of chitosan was still examined as they are utilized in medical fields, which have the most intimate connection with humans. Chitosans (having different Mw and DD; <5kDa, 65.4% DD; 5-10kDa, 55.3% DD; and >10kDa, 55.3% DD) were found to display little cytotoxicity against CCRF-CEM (human lymphoblastic leukemia) and L132 (human embryonic lung cells) (IC50>1mg/ml), from the research in 1999, conducted by Richardson et al. [36] In contrast to most reports, Carreño-Gomez et al. [37] found chitosan (hydrochloride salt) to be relatively toxic (IC50 0.21±0.04mg/ml) against B16F10 (murine melanoma) cells [37]. However, this study appears to have used solutions of the chitosan salts at the pH formed when dissolved for chitosan HCl in PBS (10mg/ml) pH=5.8. Nonetheless, despite this potential denial of harmlessness, the appreciation of the effect of the salt form of the polymer on its interaction with cells and macromolecules should be acknowledged.

Other properties of the chitosan nanoparticles to notice are particle size and surface charge and their effect on mucosal surfaces and drug release profiles. According to research conducted by Warsito et al. [38] in 2021, it was evident that smaller particle size can entrap higher concentration of therapeutic agents, improve drug stability and its bioavailability and provide sustained delivery [38]. Tsai [39] reported that mixing procedures also had an effect on the optimum temperature to obtain small CNPs. In the CNPs produced by mechanical shearing at 1000rpm, the best temperature to produce the smallest CNPs was 45 °C (145nm), followed by 4 °C (150nm) and 25 °C (163nm). However, it may be because the larger CNPs were removed during centrifugation. Thus, the smaller CNPs remained than the other CNPs formed at the temperature of 4 °C or from 25 °C. For the surface charge of the chitosan, the positive charge of chitosan enables it to attach to cells efficiently, increasing the probability of cellular uptake. Chitosan NPs are taken up by cells via different pathways and escape endosomal degradation due to the proton sponge effect. Hence, it can be concluded that the positive charge is advantageous for the chitosan NPs to be absorbed and assimilated in the human body.

## Enhancement of Oral Bioavailability

The mucus layers of the human Gastrointestinal (GI) tract are mostly made up of mucin proteins, which are often divided into highly glycosylated and non-glycosylated mucin domains. There are two types of mucins: transmembrane mucins, which are present in the cell membrane and are mostly positioned on the apical side of epithelial cells and gel-forming mucins released by mucus-producing cells [40]. The GI tract's mucus layers protect the underlying epithelial surface from hazardous chemicals and pathogens by slowing pathogen diffusion to the epithelium [41]. The mucus layer in the small intestine has a high concentration of antibacterial peptides and proteins, which eliminate germs that permeate through the top layers of the mucus layer [42]. Mucus operates as a "barrier" that must be crossed in order to ensure effective oral medication delivery since, regrettably, this protective function also lessens the diffusion of medicines (lipophilic and hydrophilic) towards the epithelium [43].

Mucoadhesion is described as the process by which interfacial forces hold two materials together for extended periods of time. If the materials are biological, the process is also known as bio-adhesion. For decades, researchers have researched mucoadhesive materials, both natural and synthetic and a few mucoadhesive drug delivery methods have been employed in FDA-approved medicines. Mucoadhesive drug delivery systems have extended stomach residence periods as a result of the interactions between the systems and the GI mucus components described above. These nanoscale or microscale medication delivery methods were primarily employed to provide tiny compounds orally [44].

Although the subcutaneous approach eliminates the first-pass effect, it can result in peripheral hyperinsulinemia [45]. Alternatively, oral insulin administration is the most convenient delivery method since it is inexpensive, safe and painless [46]. Oral insulin reaches the liver via the portal vein, lowering hyperinsulinemia and boosting cells' sensitivity to insulin, so using blood glucose more effectively. On the other hand, Oral drug delivery practices present significant challenges for the effective and efficient delivery of insulin. Poor intestinal absorption and enzymatic breakdown of high molecular

weight hydrophilic macromolecules such as insulin reduce insulin bioavailability and hepatic metabolism [47].

Therapeutic proteins like insulin experience serious threats by the enzymatic degradation within the GI tract. Chitosan, in this case, shields insulin from this enzymatic degradation, allowing the substantial amount of the active drug to reach the bloodstream. Hence, this mechanism is essential in the maintenance of the potency and bioavailability of insulin during its journey through the digestive system. Thus, the enzymatic protection provided by chitosan nanoparticles acts as a powerful advantage in utilization of oral intake of medicines. This was specifically studied by Pai et al. [48], where the chitosan was applied to the lipid nanoparticles to enhance the physical stability [48]. When applied to medications that are highly vulnerable to enzymes, such as insulin, the chitosan-lipid system can offer significant protection against enzymatic degradation.

Chitosan nano systems due to their high adhesive characteristics and naturally positively charged features. Further, the choice of chitosan-lipid system is determined by the drug's affinity for the matrix. To improve drug association efficiency, a chitosan-lipid system and a drug with comparable affinities are selected. The insulin is complexed with sodium docusate in the formulation via the chitosan-monoolein method. The process of complexation eliminates or reduces insulin's charge density, improving its affinity for lipophilic carriers. The synthesis of NPs comprising an insulin-sodium docusate complex embedded in chitosan-lipid matrix is predicted to boost the resistance of matrix and insulin to enzymatic degradation following its oral administration.

Insulin is known for its limited bioavailability when administered orally [49]. One of the advantages of chitosan is its ability to adhere to the mucosal surface when enhanced, which extends the duration of contact and enhances insulin absorption, thereby improving its therapeutic efficacy. This mucoadhesive property of chitosan makes it a valuable component in addressing the challenge of delivering insulin orally. Moreover, chitosan acts as a permeation enhancer without causing significant damage to the mucosal membrane. Chitosan has shown its effectiveness in various oral insulin formulations, including liquid mixtures, nanoparticles, and microparticles. Additionally, various derivatives of chitosan are employed as mucoadhesive and encapsulating polymers, stabilizers and permeation enhancers in these formulations, with the long-term goal of replacing the need for insulin injections [17].

Another study by Seyam et al. [50] focused on the application of insulin-loaded chitosan and chitosan derivative nanoparticles. The successful encapsulation of insulin, along with the ability to modulate size, surface charge and release profiles, presented chitosan as a promising candidate for oral insulin delivery. Compared to conventional oral insulin administration, chitosan-based nanocarriers have shown marked improvements in bioavailability and blood glucose-lowering effects. This is primarily due to chitosan's mucoadhesive properties, which prolong the residence time of the nanoparticles near absorption sites in the intestines, facilitating insulin transport through either transcellular or intracellular pathways to the bloodstream, eventually reaching the liver via the portal vein. Chitosan derivatives are also employed to enhance solubility and broaden its application as a drug delivery system while retaining its beneficial characteristics. Moreover, modifying chitosan can provide control over cargo release throughout the gastrointestinal tract and regulate the surface charge of the nanoparticles, further enhancing protection and promoting insulin absorption and bioavailability. Additionally, chitosan or its derivatives can serve as a coating for nano formulations, adding versatility in achieving specific therapeutic goals.

Chitosan's toxicity to the gastrointestinal tract is an impediment to its use as an insulin carrier. While chitosan unlocks tight connections, hazardous chemicals can easily enter the bloodstream via the paracellular route. On the other hand, excessive positive charges are trapped in the mucus layer and cannot enter the circulation. To combat retention, several negatively charged polymers, such as alginate nano compounds and polyglutamic acid compounds, have been employed to alter chitosan nanoparticles, increasing permeability across the mucus layer and oral bioavailability [51].

A variety of insulin nanocarriers, including chitosan, have recently completed clinical studies [52], most of which failed. They have drawbacks such as toxicity, limited oral bioavailability and an increase in intraindividual insulin absorption differences. Biocompatibility, biodegradability and immunological reactions should be taken into account in the near future for developing clinically meaningful insulin-loaded chitosan nanocarriers. As a result, several factors must be considered while developing innovative insulin nanocarriers, like those from chitosan derivatives. Some of the characteristics include the optimal particle size for interaction with the intestinal mucosa, the stability of the nanocarriers in biological fluids following in vivo administration, surface chemical composition, internal chemical composition and the use of targeting ligands specific to apical membrane receptors [53].

## The Gastrointestinal Tract and stability of Chitosan Nanoparticles (CSNPs)

Multiple physiological characteristics of the gastrointestinal tract, such as pH, enzymes, illnesses and micro bacteria, influence the stability and absorption of CSNP carriers. The pH of the stomach ranges from 1.5 to 3.5, making it acidic. The pH gradually rises in the small intestine, reaching 5-6 in the duodenum and 7-8 in the jejunum and terminal ileum. Meanwhile, the pH drops to a range of 5.7-6.4 in the caecum before rising again, for the last time, to a range of 6.1-7.5 in the descending colon and rectum [53]. As a result, the medications must be exposed to pH variations along the Gastrointestinal (GI) tract, which may cause drug deactivation, particularly in protein and peptide therapeutics, by modulating differential oxidation, hydrolysis or deamination [54]. The surrounding pH affects the degree of ionization (pKa) of the drug substrates, hence determining drug solubility and absorption [12]. Chitosan's amino group has a pKa of roughly 6.5, relating to its degree of N-deacetylation and is completely protonated at a

pH of 4, increasing its acidity [14]. As a result, the chitosan-loaded medication exhibits superior mucoadhesive and permeation-enhancing capabilities than the chitosan-free group in the proximal section (the stomach and duodenum), thus improving drug absorption [55]. The problem occurs when chitosan precipitates at pH levels greater than 6.5 in the jejunum, ileum, and colon, thus it has reduced adhesion to the mucus layer of the GI tract, resulting in inefficient medication absorption [56]. To overcome this disadvantage, it is possible to modify chitosan into thiolate chitosan [57].

The distal segment of the GI tract, notably the ileum and colon, is home to the human gut microbiota, which are made up of more than 1014 microorganisms [58]. The gut microbiota also plays an important role in regulating host metabolism, impacting energy balance, glucose metabolism and lipid metabolism [59]. The study of medications impacted by gut microbiota gives information on personalized medicine, which predicts drug pharmacokinetics for particular individuals. The human microbiota can influence drug-induced pharmacological and toxicological consequences. For example, the gut flora can change the oral bioavailability and action of insulin since it is vulnerable to proteolysis [60]. CSNPs and its derivatives can regulate gut microbial imbalances. For example, carboxymethyl chitosan has been shown to modify the gut microbiota in *Escherichia coli* (*E. coli*)-treated mice, influencing fat and glucose metabolism, as well as the inflammatory profile [61]. Copper-loaded Chitosan Nanoparticles (CNP-Cu) can enhance the abundance of Bifidobacterium and Lactobacillus, as CNP-Cu inhibits certain bacteria [62]. For instance, Wang et al. investigated the effects of CNP-Cu by feeding it to weaned pigs. The results revealed that the quantity of *E. coli* was significantly decreased, whereas Lactobacillus and Bifidobacterium populations increased [63].

Several processes, including simple diffusion, active transport, facilitated transport and pinocytosis, can carry drug molecules past enterocytes and reach the small intestine, which is the primary location for drug absorption. These mechanisms can occur via the paracellular or transcellular route. Drug absorption is significantly hampered by the ATP-Binding Cassette (ABC) efflux transporters, which have the ability to pump the medications out of enterocytes. P-glycoprotein (P-gp), Multidrug Resistance Protein-2 (MRP-2) and Breast Cancer Resistance Protein (BCRP) are among the drug transporter proteins found in the apical side of intestinal epithelial cells. Therefore, nanoparticle carriers like that of chitosan can, in a dose-dependent manner, reduce P-gp expression, as investigated in grass carp, where it increased norfloxacin's oral bioavailability, an antibacterial drug used to treat gram-negative bacterial infections [64].

Commercial chitosan is offered in different grades of deacetylation, molecular weight and purity. The degree of chitosan purity has a significant impact on the solubility and stability of the material in addition to its biological characteristics, including immunogenicity and biodegradability. High levels of ash and leftover proteins can make it harder for chitosan to dissolve and make it more difficult to construct chitosan-based drug delivery systems. On the other hand, enzymatic hydrolysis of the chitosan may be accelerated by microbiological contamination of the polymer. As a result, the chitosan material needs to be extremely pure and devoid of impurities, including endotoxins when applicable [65]. The relative humidity of the surrounding air has a significant impact on the amount and distribution of moisture in the chitosan substance. Water transport in chitosan material was demonstrated to follow a Fickian process at relatively low humidity levels (below 40%), but an anomalous diffusion kinetic was seen at higher humidity levels [66]. Temperature is another factor that affects the moisture content in chitosan-based systems in addition to relative humidity. It was discovered that exposure to high temperatures (40 °C) significantly reduced the moisture content of the chitosan powder (dehydration), which decreased the hardness and mechanical strength of the tablets [67] Therefore, maintaining high purity and controlling humidity and temperature are essential for the stability and effectiveness of chitosan-based drug delivery systems.

## Conclusion

The paper aimed to investigate the synthesis methods, key properties and bioavailability-enhancing mechanisms of chitosan nanoparticles, with a particular focus on their mucoadhesive capabilities and protective roles in the gastrointestinal tract and how they are currently advancing in the healthcare and nanotechnology market. Based on the secondary information gathered from a comprehensive literature review, several crucial findings were determined. The mucoadhesive capabilities and protective roles were found to be heavily reliant on temperature and the chemical environment, which suggest that the NPs might not be fully operationalizable yet. Nonetheless, the ongoing research regarding chitosan suggests a hopeful future in the application of chitosan in the oral administration of drugs, especially with the increasing thermal and chemical stability of these compounds. Multiple studies have indicated successful syntheses of CSNPs in a variety of shapes and sizes, each suited to accomplishing a specific task. In addition to enhancements in bioavailability, CSNPs have shown improvements in absorption rates and biocompatibility, indicating trends of growth in the pharmaceutical industry. Most importantly, multiple methods to produce these chitosan nanoparticles at a cheaper price were a pivotal factor in determining the chitosan nanoparticle's greater availability in upcoming years. Hence, from this research, multiple drawbacks and limitations of the current status of chitosan nanoparticles were identified, implying the difficulty for it to be utilized in the oral administration of drugs; however, multiple pieces of research were found displaying an increasing interest and development of the chitosan nanoparticles, suggesting a significant expansion in the pharmaceutical market for the use of targeted drug delivery. Despite current limitations in oral drug administration, ongoing research and growing interest suggest that chitosan nanoparticles hold significant potential for expanding targeted drug delivery in the pharmaceutical and nanotechnology market.

## Author Contributions

Ronith Lahoti, Taehoon Kim and Aidan Weaver contributed to writing, researching, formatting and editing the manuscript. Rontih focused on the introduction, gastrointestinal tract and stability, and the enhancement of oral bioavailability, while Taehoon and Aidan worked on the synthesis and properties, and the enhancement of oral bioavailability. Taehoon and Aidan also contributed to the conclusion. Additionally, Aidan focused on the writing of the abstract. Ronith oversaw the coordination of the writing process. All authors provided critical feedback and helped shape the research and manuscript.

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