A comprehensive review of oral chitosan drug delivery systems

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Review Article

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A comprehensive review of oral chitosan drug delivery systems: Applications for oral insulin delivery

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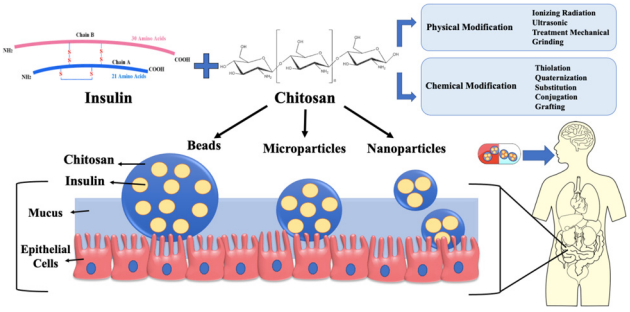
Abstract: Pharmaceutical scientists have long struggled to develop reliable and efficient systems of administering insulin orally due to multiple barriers, including stomach acidity, enzymatic degradation, and mucus barriers. However, various strategies were developed to avoid insulin degradation in the gastrointestinal tract (GIT) and promote membrane permeability and biological activity. Among these strategies, chitosan polymer-based carriers are widely researched due to their ability to protect insulin in the alimentary canal and deliver it effectively through the intestinal mucosa, improving its bioavailability. To improve chitosan properties, chemical and physical modifications have been developed, and recently, nanoparticles, microparticles, and beads of chitosan exhibited potential systems for oral insulin delivery (OID). This review facilitates an outline of the types of diabetes mellitus, insulin biosynthesis, and gastrointestinal barriers against oral insulin. Moreover, the limitations of subcutaneous insulin delivery and alternative routes of administration are also discussed. As an ideal and most convenient oral administration route, the challenges of safe insulin delivery through the GIT and strategies to elevate its bioavailability are highlighted. In addition, this review focuses on recent advancements in chitosan based carriers for OID and their potential future applications.



Graphical Abstract

Keywords: diabetes, oral insulin delivery, chitosan, colon delivery, controlled release

1 Introduction

Diabetes mellitus (DM) is a chronic endocrine disorder characterized by increased blood glucose levels due to impaired insulin synthesis caused by reduced or failure in pancreatic insulin secretion and/or low cell sensitivity toward insulin [1]. DM can primarily be diagnosed by the high level of blood or plasma glucose. Diabetes can also be detected if fasting plasma glucose level of 126 mg/dL or a 2 h plasma glucose level of 200 mg/dL with nonspecific symptoms are observed [2]. Diabetes can manifest as a contributing factor for triggering syndromes such as Turner syndrome, Prader-Willi syndrome, Friedreich ataxia, Alström syndrome, Klinefelter syndrome, Bardet-Biedl syndrome, Berardinelli-Seip syndrome, and Down syndrome [3]. Also, stress, war trauma, glucocorticoids, surgery, thiazides, starvation, infections, high levels of epinephrine, glucagon, and growth hormone are additional triggers that may lead to an autoimmune reaction that results in DM [3].

According to the International Diabetic Federation (IDF), 643 million adults will have diabetes by 2030, up from an estimated 537 million in 2021 [4]. The high

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complications and prevalence of diabetes are responsible for around three million deaths yearly worldwide, according to the World Health Organization [5]. Subcutaneous injections are still the most common approach to deliver insulin regularly. However, this route has several disadvantages, including reduced patient compliance due to pain, needle phobia, hypoglycemic episodes, and allergic reactions [6]. The convenience of administering insulin through the oral route is frequently acknowledged as the preferred method, although it confronts major obstacles of limited intestinal penetration and low insulin stability in the gastrointestinal tract (GIT). Thus, during the past 10 years, the emphasis on encapsulating insulin in polymer-based carriers has been proposed as a viable technique for improving insulin oral bioavailability by overcoming the orally associated limitations [7]. In addition, therapeutic proteins and peptides are increasingly being delivered orally using several hydrophilic mucoadhesive vehicle types like nanoparticles (NPs), microparticles (MPs), and beads as delivery systems with different advanced preparation methods [8–11]. These carriers showed improved strategy for attaching to the gut wall and increasing the residence time in the intestine, thereby boosting the drug's bioavailability, offering advantages over other drug delivery methods [12,13]. As a natural polysaccharide polymer, chitosan is considered the most common mucoadhesive polymer for delivering proteins and peptides orally. It is non-toxic, inexpensive, biodegradable, biocompatible, easy to process, and can be digested by colonic microbial enzymes. Furthermore, the availability of amine groups in its chemical structure makes it a perfect candidate for various modifications, conferring it a superior polymer compared to other polysaccharides [14,15].

Chitosan-based oral delivery systems, like NPs, MPs, and beads, have been explored as a potential option for delivering insulin orally in recent years. This comprehensive review will address the challenges and transport mechanisms associated with developing such systems for oral insulin delivery (OID). Moreover, the aim of this review is to provide a detailed description of the use of chitosan in designing OID systems, focusing specifically on recent strategies to enhance chitosan through various modifications. In addition, methods used for preparing chitosan carriers are discussed in detail.

2 DM

2.1 Type 1 DM (T1DM)

T1DM accounts for 5–10% of people with diabetes, characterized by damaged pancreatic beta cells and reduced or even terminated insulin production (Figure 1). It results from the cellular-mediated death of pancreatic beta-cells, which can be due to autoimmune or environmental-related factors [16]. The autoimmune destruction includes autoantibodies of islet cells, insulin, glutamic acid decarboxylase (GAD65), and tyrosine phosphatases IA-2 and IA-2β [17,18]. Studies on environmental-related factors have shown that diabetes prevalence varied among people of the same ethnic group living in different geographical areas (*e.g.*, Finland *vs* Estonia). In Estonia (Baltic area), the risk of developing T1DM was one-third of that in Finland. In addition, exposure to antigenic substances at a young age is also thought to play an influential role in disease development [19]. For blood glucose management and control in type 1 diabetic patients, several daily exogenous insulin injections are necessary [6,20].

2.2 Type 2 DM (T2DM)

T2DM is also one of the most frequent metabolic disorders globally, and its occurrence is commonly caused by two main factors: (1) failure to respond to insulin by insulin-sensitive tissues and (2) impairment of insulin secretion and action by pancreatic β-cells (Figure 1) [21,22]. Impaired resistance to insulin action occurs when target cells cannot respond to the circulating insulin [23]. T2DM patients also have a significant risk of infections brought on by an abnormal immune system, impaired glucose control, and diabetic neuropathy. These are either common mild infections such as external otitis, cystitis, pneumonia, enteric infections, appendicitis, and peritonitis or rare severe infections like emphysematous pyelonephritis [24]. Regarding signaling insulin pathways, T2DM is a complex disorder influencing several metabolic pathways [25]. When glucose reaches physiological amounts, it stimulates the β-cells to produce and release insulin, facilitating glucose uptake by muscle, adipose tissue, liver, and brain. Insulin also stimulates the synthesis of glycogen, proteins, and lipogenesis while suppressing hepatic gluconeogenesis production [26]. Physiologically, insulin has various hormonal effects in addition to its well-known characteristic of reducing blood sugar, explaining its impact on different tissues. The first step in the insulin signaling process is binding to the receptors that initiate a sequence of phosphorylation processes where intracellular protein substrates are activated to induce signaling cascades. Later, phosphatidylinositol 3-kinase (PI3K) triggers the activation of protein kinase B (PKB), known as AKT. In target cells, except for hepatocytes, which mainly express the non-insulin-regulated glucose transporter 2 (GLUT2), translocation of GLUT4 to the plasma

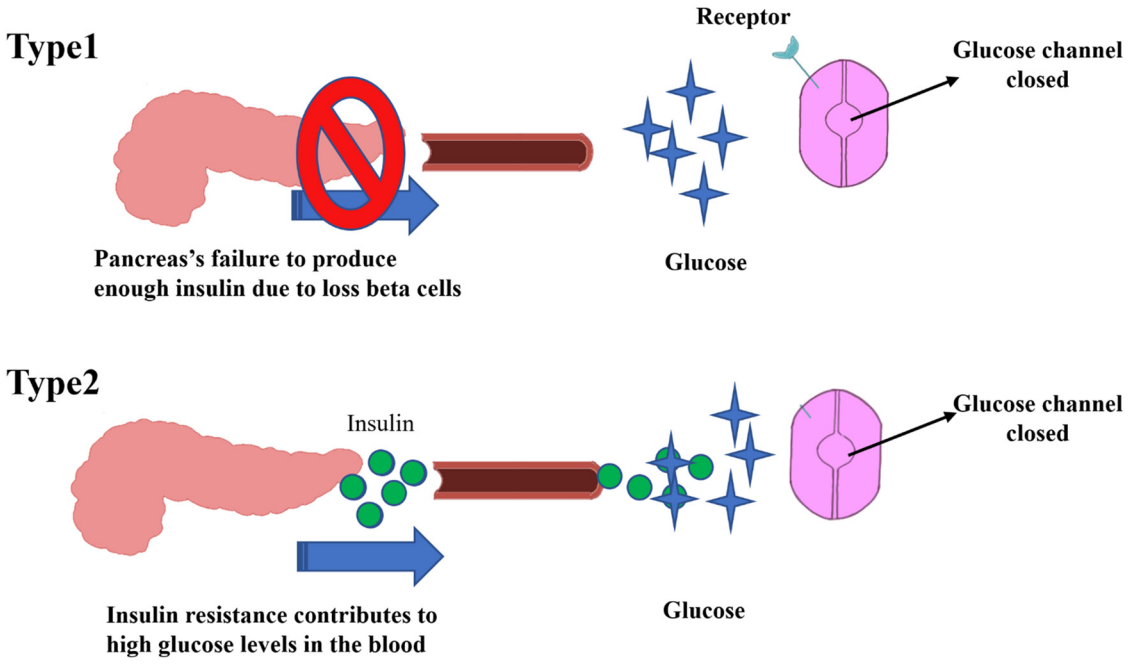

Figure 1: Schematic presentation of insulin fate in T1DM and T2DM.

membrane, glycogen synthase, and a variety of other enzymes are then triggered through the action of insulin. Mitogen-activated protein kinase activity, which encourages protein translocation, gene expression, and cell proliferation, also impacts the insulin signaling pathway. Ultimately, the metabolism of lipids, proteins, and carbohydrates is significantly regulated by insulin [21].

2.3 Insulin biosynthesis

Insulin is synthesized by converting insulin mRNA into preproinsulin, a single-chain molecule that serves as an inactive insulin precursor. The composition of preproinsulin consists of a single peptide that includes insulin B-chain, C-peptide, and insulin A-chain (Figure 2) [27]. Once the single-chain molecule is secreted to the endoplasmic reticulum, proinsulin is created by eliminating the signal peptide through signal peptidase. Proinsulin comprises three unique chains consisting of an amino-terminal B chain, a C-peptide, and a carboxy-terminal A chain, forming 86 amino acids. Proinsulin is recognized as the prohormone precursor to mature insulin. Proinsulin is transformed into insulin in the endoplasmic reticulum by cutting out the C-peptide by specific endopeptidases (Figure 2) [28]. Finally, the C-peptide and insulin are transported to the Golgi for packaging into secretory granules and stored in the cytoplasm [29].

3 Current insulin administration

The GIT has a hostile and harsh environment that can cause insulin degradation [30]. To avoid this degradation, insulin is usually administered through subcutaneous injections that offer rapid onset of action and high bioavailability [31]. Patients commonly receive insulin injections directly into the subcutaneous fat layer with a lower absorption rate due to reduced vascularity. Although there has been a significant improvement in various techniques for subcutaneous insulin injections, this technique can still lead to several issues, including patient discomfort, poor compliance, nonadherence, infections, lipid deposits at the injection site, and local hypertrophy [32]. In addition, as this route is invasive, the subcutaneous injection formulations might not be the best choice when multiple injections are needed per day. In fact, it is reported that hypoglycemia can occur when multiple injections are given leading to poor control of blood glucose and thus putting patients at high risk of low sugar complications. Therefore, alternative administration routes, such as oral, buccal, nasal, peritoneal, and transdermal, have recently gained significant attention [33,34].

4 Non-oral insulin delivery

Insulin has various duration effects, including rapid, short, intermediate, and long-acting [35]. Also, different insulins,

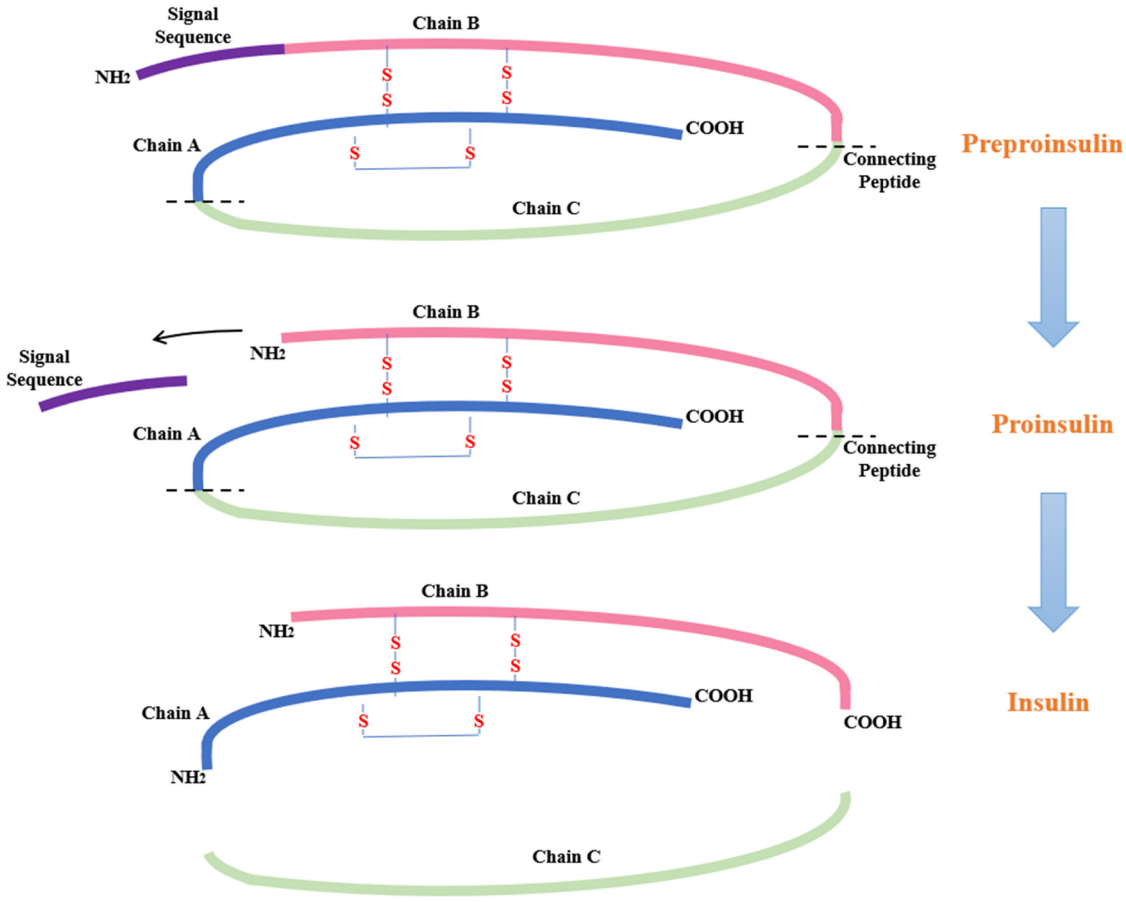

Figure 2: Biosynthesis of insulin.

including basal, prandial, and premixed, are administered through a pump, syringe, pen, and prefilled pen. There are long-acting analogues for basal insulins (first generation: detemir and glargine 100 I/mL; second generation: glargine 300 U/mL and Degludec) as well as human intermediate-acting insulin (neutral protamine Hagedorn insulin, for example). The basal short-and rapid-acting insulin analogues (*e.g.*, Aspart, Lispro, and Glulisine) and prandial insulin require frequent daily injections. The pre-mixed insulin analogues combine slow- and rapid-acting insulin in varying ratios to replicate the prandial and basal effects of insulin production in a single dose, to reduce the dose frequency of intermediate/rapid-acting insulin, hence improving the patients' convenience. However, combining rapid and intermediate-acting insulins is significantly challenging as basal insulin is not miscible with other insulins; thus, part of rapid-acting insulin should contain protamine to convert it into intermediate-acting insulin [36].

Besides injection, non-OID, such as pulmonary and nasal, was developed, considering the large surface area of the respiratory, pulmonary, and nasal pathways. However, there are some safety concerns as these routes can allow the entry of exogenous allergens into the lung and have an irreversible alteration of the epithelial cell membrane [37]. The nasal administration possesses a highly vascularized absorption region (150 cm^2) that allows rapid circulatory drugs [38]. This facilitates direct protein transfer into the blood circulation, thus avoiding first-pass hepatic and gut metabolism [39]. Nevertheless, a local burning sensation in the nose is a common side effect after administering the drug through the nasal. Although intranasal insulin was widely employed in clinical trials, only a few studies reported satisfactory results [40].

Transdermal insulin can bypass the enzymatic and chemical degradation in the GIT. This approach can also provide a sustained release with maintained therapeutic concentration for an extended period, allowing for better glycemic control. However, the skin's protective properties are still the main challenge for transdermal insulin delivery [41]. Finally, buccal mucosa has received considerable attention due to its visibility, resilience, saliva-protected, and highly vascular properties. However, buccal administration

has numerous intrinsic limitations, one of which is the limited permeability of neutral lipids across the surface layers of the epithelium [42].

5 Oral insulin delivery

Although the subcutaneous route overcomes the first-pass effect, it can lead to peripheral hyperinsulinemia [43,44]. Alternatively, oral insulin administration is the most convenient delivery route as it is cost-effective, safe, and painless [45]. Oral insulin passes the liver *via* the portal vein, reducing hyperinsulinemia and increasing cells' sensitivity to insulin, hence utilizing blood glucose more effectively [44]. Moreover, the GIT cavities offer a large surface area for enhanced drug absorption that mimic the physiological insulin pathway, improving glucose homeostasis [46]. Furthermore, OID can improve insulin portal levels while lowering peripheral hyperinsulinemia linked to retinopathy and neuropathy in other administration routes [18,47]. However, OID is highly challenging in delivering insulin effectively and efficiently, which is the primary research highlighted in recent years [48]. For high molecular weight hydrophilic macromolecules like insulin, poor intestinal absorption and enzymatic degradation negate insulin bioavailability and hepatic metabolism [49]. Although many approaches are developed to deliver insulin orally, the absorption rate and the capacity to penetrate the gut lining vary significantly [50].

5.1 Barriers to OID in the stomach and small intestine

As shown in Figure 3, the stomach, which contains high gastric acid, comprises the most significant chemical barrier to insulin absorption. Gastric acid is a digestive fluid containing hydrochloric acid secreted by the parietal cells of gastric glands in the stomach, resulting in a highly acidic environment (pH 1~2). Moreover, the pepsin enzyme in the stomach degrades and proteolyzes the free insulin. As a result, shielding insulin from both acids and enzymes is a significant step in aiding to deliver oral insulin efficiently [51]. Among several available strategies, pH-triggered release approaches are used to overcome stomach acidity. pH-responsive carriers such as hydrogel demonstrated enhanced drug oral delivery and controlled release in the intestine [52]. Pancreatic enzymes like lipase, elastase, chymotrypsin, trypsin, and carboxypeptidases (A and B) can degrade proteins transiting the small intestine and lumen [53]. The most common strategy for suppressing these enzymes is by including

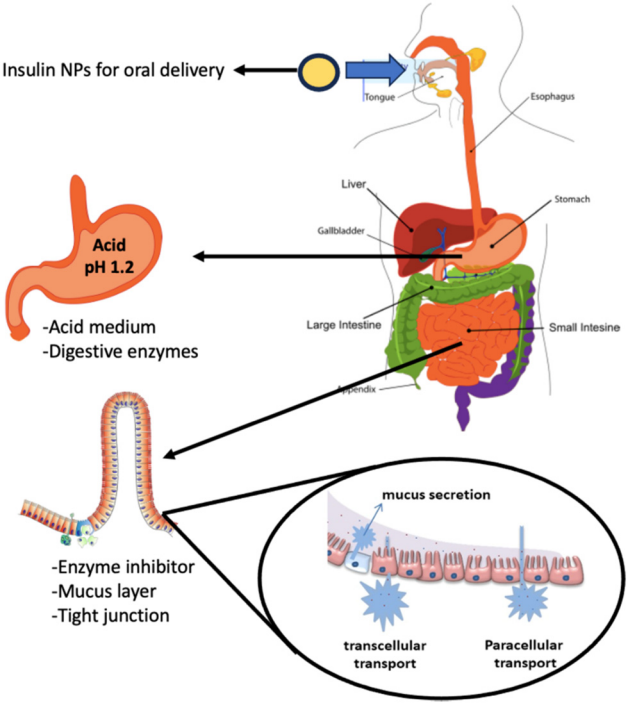

Figure 3: The GI barriers for OID.

enzyme inhibitors in drug formulation, such as non-amino acids, amino acids, modified amino acids, peptides, and modified peptides that inactivate target enzymes by reversibly or irreversibly attaching to the particular sites of the enzymes [54].

Furthermore, delivering insulin orally faces physical barriers such as the mucous layer, intestinal epithelium and tight junctions. Mucus is the first obstacle against polypeptides that can serve as diffusional and enzymatic barriers. Mucus, an adhesive and viscoelastic hydrogel covers the GIT epithelial cells and exhibits a strong capacity to trap unknown particles by physicochemical forces like hydrogen bonds, electrostatic interactions, and hydrophobic forces. Eventually, these trapped particles are removed from the body *via* a natural mucociliary clearance pathway [55]. Also, mucus interacts electrostatically with positively charged drugs and proteins due to its negative charge [47].

The intestine has a layer of epithelial cells protected by a mucus layer, the major component of the intestinal mucosal barrier. Before oral insulin reaches the bloodstream, it must pass through the intestinal epithelium *via* two pathways, transcellular or paracellular. Most oral medications are absorbed transcellular, which involves intermembrane transport, endocytosis fusion, and adsorption [56]. At the same time, hydrophilic molecules are preferentially transported by the paracellular pathway regulated by tight junctions between the epithelial cells. The absorption

of these molecules is regulated by the junctional complexes, including adherens junctions (AJs), desmosomes, and tight junctions (TJs). TJs consist of peripheral membrane proteins (zonula occludens (ZO)-1, ZO-2), regulatory proteins, and transmembrane proteins (occludins and claudins), where TJs are the apical-most adhesive complexes that primarily close the intercellular space. AJs are required for their assembly and are found below the TJs. Together with desmosomes, it provides strong adhesive bonds to retain the integrity of the epithelium. AJs and TJs are connected to the prejunctional ring of myosin and actin that allows the control of junctions *via* the cytoskeleton [57].

6 Oral delivery of insulin *via* colon targeting

As OID faces numerous challenges, the colon-targeted protein drug delivery systems were intensively investigated due to the neutral pH (~7.5), minimal proteolytic activity, and prolonged residence duration [58]. The large intestinal tract comprises the cecum, colon, rectum, and anus, with the colon being the largest at roughly 1.5 m in length. Generally, the high water-absorbing capacity of the colon confers it more viscous than the upper GIT parts. Besides, the lumen walls of the colon are covered by a thick mucus layer that consists of mucin glycoprotein, lipids, water, and inorganic ions, which reduce drug dissolution and absorption [59]. Therefore, the colon poses a potential target site for the oral delivery of insulin.

6.1 Challenges in colon targeting insulin delivery

Although the colon delivery system can potentially be used for macromolecule administration, several obstacles complicate oral drug delivery to the colon. For example, the burst drug release, drug degradation in the stomach, the variation in pH in different GIT regions, mucus entrapment, and systematic jejunum [60] make the colon-target delivery system more challenging. The human digestive tract is complex, and several factors should be considered in designing a targeted colonic drug delivery system, including the pH, mucus barrier, and colonic microbiota [61].

6.2 pH

Although varying pH values along the GIT can complicate colon-targeted delivery systems, the differences in pH ranges can help control drug release time. Colon-targeted delivery systems use a pH-sensitive polymer as a coating agent to cover the entire drug surface that is insoluble in acidic pH but soluble in neutral pH or slightly alkaline [62]. pH-sensitive polymers enable shielding the drug while in the upper region of the GIT and suppressing the encapsulant release before approaching the colon. The coating may consist of single or multiple enteric polymers of pH-dependent or a mixture with pH-independent polymer [61]. In addition, a formulation based on one or a pair of enteric polymers with different pH-dependent solubility profiles can be formulated for colon drug delivery [63].

6.3 Mucus barrier

Another barrier that influences the absorption of drugs in colon delivery is the hydrogel layer, composed of large mucin glycoproteins, known as mucus. Mucus protects the epithelium from mechanical damage, traps and prevents infections from accessing the epithelial cells, and lubricates the chyme. Conventional drug delivery methods can also stick to the GI mucosal layers before being removed in the feces, shortening the sustained local drug release period and resulting in unsatisfactory therapeutic effects [60]. However, the mucus can benefit specific delivery systems by prolonging the drug residence time [64]. While high molecular weight proteins and peptides may not effectively penetrate through the mucus, drug molecules with hydrophilicity, net-neutral surface charge, and smaller hydrodynamic size can negate adhesion, resist mucosal enzymatic action, and circumvent steric hindrance [65].

6.4 Colonic microbiota

In the digestive tract, the anaerobic and aerobic bacteria constitute the most significant number of bacteria in the colon. These bacteria have around 400 types with a concentration of 1,000 CFU/mL [66] that produce some biomolecules and metabolize drugs [67]. Polysaccharides are prone to anaerobic bacterial metabolism in the colon while resisting the intestinal and gastric enzymes. Moreover, colonic enzymes can mediate drug biotransformation that results in inactive, active, or harmful metabolites, whereas bacterial drug metabolism can cause toxicity. An active metabolite formed by colonic drug metabolism is a typical "prodrug approach" for colon-specific drug delivery systems [60]. As a result, while designing a medication delivery

system for the colon, it is critical to consider therapeutic formulations based on bacterial enzymes [68].

7 Polymers-based carrier for OID

Over the last decade, several research studies based on polymeric carriers to improve the delivery of insulin orally have been conducted [69]. Various polymers were investigated to determine the possible applications, emphasizing natural polymers. Natural polymers are preferred over artificial counterparts regarding biocompatibility, accessibility, and ease of modification. Hence, various functional groups can be integrated into the native natural polymers, providing additional physicochemical properties.

Monosaccharide chains are linked by hydroxyl, carboxyl, and amino groups to produce polysaccharides, typically extracted from organic materials, marine plants, and exogenous bacterial metabolites. Polysaccharides are hydrophilic, stable, non-toxic, readily biodegradable, and can encapsulate, immobilize, and release various active substances under controlled conditions [70]. Polysaccharides such as chitosan, pectin, alginate, starch, and dextran were extensively employed as encapsulation matrices [71,72].

In recent years, introducing polymeric NPs/MPs as carriers to deliver insulin orally has gained significant interest [73]. Numerous biodegradable and non-biodegradable polymers were studied, but the latter pose issues with toxicity, removal challenges, and the inability to achieve sustained release of insulin. Biodegradable polymeric particles separate the drug from the surrounding medium, shielding the peptide from peptidases and facilitating enterocyte uptake. After oral administration, polymeric particles gradually degrade depending on the nature of the polymer, providing a sustained and controlled drug release.

8 Chitosan-based polymer for oral insulin drug delivery

The mucoadhesive polymer-based drug delivery can boost absorption and bioavailability by extending drug retention at the absorption site. Ideally, the mucoadhesive polymer should attach to the mucosa before penetrating the mucus layer. Protein drug delivery systems based on mucoadhesive property have been developed using polymers such as chitosan, alginate, and many others [74]. Other properties of these polymers, including gastro-resistance, degradability by colonic microbiota, and pH responsiveness, provide micro- and nano-based drug delivery systems several advantages in overcoming barriers to oral drug administration and targeting specific absorption sites [75]. Chitosan is considered one of the most effective polymers in developing oral drug administration systems among all available mucoadhesive polymers. Chitosan is a positively charged, non-toxic, biodegradable, biocompatible, and mucoadhesive polymer produced by the hydrolysis of crab or shrimp chitin [10].

8.1 Physicochemical properties of chitosan

Chitosan, a polysaccharide, comprises repeating D-glucosamine and *N*-acetyl-D-glucosamine blocks (Figure 4). Following deacetylation, each chitosan subunit includes a primary amine group (pK_a = 6.5) and two hydroxyl groups that can be conveniently modified depending on the purpose of application. At pH < 6.5, chitosan amine groups are protonated to NH^{3+}, conferring chitosan cationic and soluble in an acidic medium. The degree of deacetylation and molecular weight of chitosan significantly impact its solubility [76].

Chitosan gains its mucoadhesive properties through ionic interactions between its cationic groups and the anionic nature of the mucous layer. These characteristics promote its adhesion into the GITs, retaining the encapsulant for a prolonged time to reach a sustained release profile [77]. The mucoadhesive property is vital in developing drug delivery systems that improve drug targeting, controlled drug release, effectiveness, and bioavailability [78]. Furthermore, chitosan can form gels in acidic pH that can be utilized as carriers for slow drug release. Moreover, it can be cross-linked with covalent bonds through the reaction between the negative phosphate groups of sodium triphosphate (TPP) (non-toxic) and the amino groups of chitosan [79]. Pharmaceutically, chitosan is employed in several applications, such as clinical biomedicine, drug delivery systems, tissue engineering, and taste masking [80]. Recently, chitosan has attracted wider attention in developing micro- and nano-carriers as oral delivery systems due to the modifiable physicochemical characteristics of its backbone.

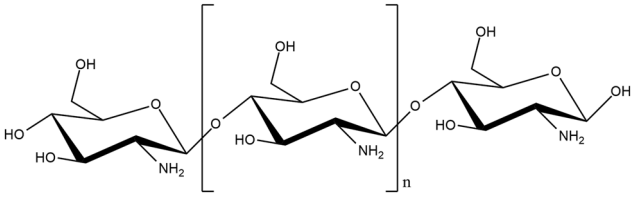

Figure 4: Chemical structure of chitosan.

8.2 Drug release from chitosan matrix

Recent studies have employed chitosan in drug delivery systems for various applications as described in (Table 1). Like other dosage forms, chitosan-based drug delivery systems rely on the physicochemical properties of the encapsulant. In addition, drug delivery in these systems depends on chitosan properties such as swelling, adhesion to mucus layer, and gel-forming ability in different body fluids with various ion concentrations and pH, as well as the presence of excipients and co-polymers in drug formulations. The release of encapsulated drug from chitosan hydrogel is affected by diffusion, swelling, erosion, and biodegradation [81].

A cationic polymer like chitosan can promote polyelectrolyte complexation with an anionic polymer like pectin to control the drug release with the required protection. In coacervation with pectin, chitosan helps encapsulate anionic drugs like bovine serum albumin. The prepared particles showed an enhanced pH-sensitive drug release profile, whereas the results of the animal studies on rats showed that 72.6% of physiologically active anti-A/B toxin immunoglobin of egg yolk (IgY) was released preferentially in the colon [82]. Several studies demonstrated the colon-targeting capability of chitosan-calcium pectinate microbeads compositions with a limited drug release in the stomach and small intestine and a controlled release in the colon [83]. Hence, a chitosan-based hydrogel system is a promising candidate for developing oral colon insulin delivery [84].

8.3 Chitosan limitation

The increased swelling of chitosan in aqueous environments can cause a rapid premature drug release. Moreover, chitosan-intrinsic properties, such as low mechanical resistance and high matrix porosity, can impact its application in drug delivery [85]. Furthermore, chitosan has weak acid resistance, and its solubility is poor in physiological fluids (pH around 7.4) due to its weak basic nature with pK_a between 6.2 and 7 [86]. To overcome these limitations, chitosan is commonly derivatized and/or modified physically or chemically [81].

9 Applications of chitosan systems for OID

The management of diabetes, a global health concern, has long been dominated by traditional insulin administration methods, primarily injections [87]. However, the search for non-invasive alternatives has led to significant advancements in drug delivery systems. The advancements in OID through chitosan systems represent a significant breakthrough in the landscape of diabetes management. The multifaceted contributions of chitosan, encompassing biocompatibility, mucoadhesive properties, controlled release mechanisms, and protection against enzymatic degradation, position it as a versatile and effective platform for ensuring successful oral delivery of insulin. As a protein drug, the orally administered insulin must pass through many physiological barriers, such as GIT, mucus layer, intestinal epithelium, then finally reach the circulatory system [88]. Thus far, oral absorption of insulin remains a major scientific challenge. First, oral insulin must be effectively transported along the GIT tract without being degraded by acidic conditions in the stomach and proteases in the GIT. Second, insulin is a hydrophilic protein, which is difficult to be encapsulated in a hydrophobic macromolecular carrier. Third, the bioavailability of untreated insulin is extremely low due to the first-pass effect in the liver. Additionally, transit time is another factor that affects oral delivery and bioavailability of drugs to the colon. The normal transit time in the small intestine is approximately 4 h, with an inter-individual variability of 2–6 h; that of colon, however, is relatively variable, ranging from 6 to 70 h [89]. Chitosan polymer and its derivatives were used in several OID systems due to their properties such as mucoadhesive property, controlled release, and protection against enzymatic degradation.

9.1 Mucoadhesive property

Chitosan's mucoadhesive properties as illustrated in Figure 5 add another layer of sophistication to its role in OID [90]. In the GIT, the mucosal lining presents a formidable barrier that drugs must overcome for efficient absorption. Chitosan's mucoadhesiveness allows the encapsulant to interact with the mucosal layer, extending the residence time of the drug delivery system. This positive impact is attributed to its ability to open the tight junctions between epithelial cells, facilitating the transport of insulin through well-organized epithelial layers. Insulin is a peptide hormone known for its poor oral bioavailability, the ability to adhere to the mucosal surface is particularly advantageous [91]. The prolonged contact facilitated by chitosan enhances the absorption of insulin, contributing to its therapeutic effectiveness. This mucoadhesive feature positions chitosan as a key player in addressing one of the longstanding challenges in OID. Importantly, chitosan serves as a permeation enhancer

Table 1: Chitosan applications for colon targeted drug delivery

Formulation	Active pharmaceutical ingredients (APIs)	Delivery system	Approaches for CDDS	Degradation mechanism	Ref.
Chitosan/pectin	Curcumin	Modified citrus pectinate-chitosan NPs	Drug delivery to the colon using a pH-sensitive polymer-coated carrier	Mucoadhesiveness	[123]
Chitosan/kappa-carrageenan/alginate	5-Fluorouracil	pH-sensitive bilayered chitosan/kappa-carrageenan microbeads/alginate	Drug delivery to the colon using a pH-sensitive polymer-coated carrier	pH-responsive	[124]
Chitosan/alginate	Interleukin-1RA	Chitosan/alginate microcapsules	Drug delivery to the colon using a pH-sensitive polymer-coated carrier	pH-responsive	[125]
Chitosan/pectin	Insulin	Chitosan-pectin NP colon-specific drug delivery	Drug delivery to the colon using a pH-sensitive polymer-coated carrier	pH-responsive	[126]
Chitosan-heparin	Oligonucleotides	Chitosan-heparin NP colon-specific drug delivery	Drug delivery to the colon using a pH-sensitive polymer-coated carrier	pH-responsive	[126]
Fucoidan/chitosan	Quercetin	Fucoidan-chitosan NP colon-specific drug delivery	Drug delivery to the colon using a pH-sensitive polymer-coated carrier	pH-responsive	[127]
Alginate-chitosan	BSA	Alginate-chitosan NP colon-specific drug delivery	Microbially triggered drug delivery to the colon	Enzymatic	[128]
Chitosan	Resveratrol	NP colon-specific drug delivery	Microbially triggered drug delivery to the colon	Enzymatic sensitive	[129–131]
Pectinate-chitosan	Curcumin	Modified citrus pectinate-chitosan nanoparticle	Microbially triggered drug delivery to the colon	Enzyme sensitivity and mucoadhesiveness	[123,132]
	Resveratrol	Chitosan-zinc-pectinate-polyethene glycol NPs	Drug delivery to the colon using a pH-sensitive polymer-coated carrier		
Thiolated chitosan	Sitagliptin	NP colon-specific drug delivery	Drug delivery to the colon using a pH-sensitive polymer-coated carrier	Mucoadhesiveness	[133]
Chitosan	Insulin TDF	NP colon-specific drug delivery	Drug delivery to the colon using a pH-sensitive polymer-coated carrier Microbially triggered drug delivery to the colon	Enzymatic, pH-responsive	[130]
Chitosan + mucin	Insulin	NP colon-specific drug delivery	Drug delivery to the colon using-sensitivities polymer-coated carrier	Mucoadhesiveness	[134]
Chitosan/ alginate	Naringenin	NP colon-specific drug delivery	Drug delivery to the colon using a pH-sensitive polymer-coated carrier	pH-responsive	[135]
PEGylated chitosan	Rosuvastatin	NP colon-specific drug delivery	Drug delivery to the colon using a pH-sensitive polymer-coated carrier	pH-responsive	[136]
Succinyl chitosan/ alginate	Quercetin	NP colon-specific drug delivery	Drug delivery to the colon using a pH-sensitive polymer-coated carrier	pH-responsive	[137]
Chitosan/ alginate	Lovastatin	NP colon-specific drug delivery	Drug delivery to the colon using a pH-sensitive polymer-coated carrier	pH-responsive	[138]
Chitosan anti-biopeptides	Lovastatin	NP colon-specific drug delivery	Microbially triggered drug delivery to the colon	Enzymatic responsive	[139]
Chitosan-modified	Curcumin	NP colon-specific drug delivery	Ligand/receptor-mediated drug delivery system	Epithelium	[140]
Chitosan/alginate	Capecitabine	Chitosan succinate-sodium alginate beads	Drug delivery to the colon using a pH-sensitive polymer-coated carrier, microbially triggered drug delivery to the colon	pH-responsive, enzyme-sensitive, and mucoadhesiveness	[141]

(Continued)

Table 1: *Continued*

Formulation	Active pharmaceutical ingredients (APIs)	Delivery system	Approaches for CDDS	Degradation mechanism	Ref.
Alginate/chitosan/Konjac glucomannan	Ciprofloxacin	Chitosan coated konjac glucomannan/ sodium alginate/graphene oxide microspheres	Drug delivery to the colon using a pH-sensitive polymer-coated carrier, microbially triggered drug delivery to the colon	pH-responsive, enzyme sensitive, and mucoadhesiveness	[124]
Chitosan/nutriose	Quercetin	PEG-containing vesicles coated with chitosan/ nutriose	Drug delivery to the colon using a pH-sensitive polymer-coated carrier, microbially triggered drug delivery to the colon	Enzyme sensitivity and mucoadhesiveness	[142]

without causing significant harm to the mucosa. Chitosan has demonstrated effectiveness in various oral insulin formulations as a transmucosal delivery polymer, with the goal of eliminating the need for injections over time. Both chitosan and insulin have been incorporated into various forms such as liquid mixtures, NPs, and MPs and others. Additionally, a range of chitosan derivatives are utilized as mucoadhesive, encapsulation polymers, stabilizers, and permeation enhancers [92–96].

Chitosan, a positively charged polymer, holds promise for OID due to its modifiable nature through various chemical reactions. However, in its original form, chitosan's effectiveness as an absorption enhancer for insulin in the intestinal tract can be compromised. To address this, several chemical modifications such as chitosan thiolation, conjugation, and grafting have been applied. While possessing excellent mucoadhesive characteristics, they also exhibit permeation-enhancing effects, the capacity to inhibit efflux pumps, and the ability to form *in situ* gels [97–99].

A previous conducted study involved chitosan NPs suspended in deionized water for OID. The obtained SPECT/CT images highlighted a notable presence of chitosan nanoparticles, marked with 99mTc, persisting in the stomach for an extended duration post oral administration. To address potential insulin release from chitosan NPs, a strategic step was taken. The chitosan NPs were subjected to lyophilization, and the resulting dried NPs were encapsulated in a gelatin capsule featuring an enteric polymer coating (Eudragit L100-55). This enteric polymer exhibits pH sensitivity, ensuring resilience in the acidic gastric environment while readily dissolving in the mildly acidic to neutral conditions of the small intestine. This ingenious approach serves a dual purpose – preventing premature insulin release in the stomach and promoting enhanced absorption on the small intestine's surface. Ultimately, this methodology aims to maximize the bioavailability of insulin delivered through chitosan NPs [100,101].

9.2 Controlled release mechanisms

Chitosan's versatility is further evident in its ability to be tailored for controlled release, a critical aspect of optimizing OID [102]. Various formulation techniques, including microencapsulation and NP design, allow researchers to modulate the release kinetics of insulin from chitosan-based systems. Controlled release mechanisms are paramount for achieving sustained and prolonged insulin delivery, mimicking the physiological secretion profile of endogenous insulin. This not only enhances therapeutic efficacy but also contributes to better glycemic control for individuals with diabetes. The controlled

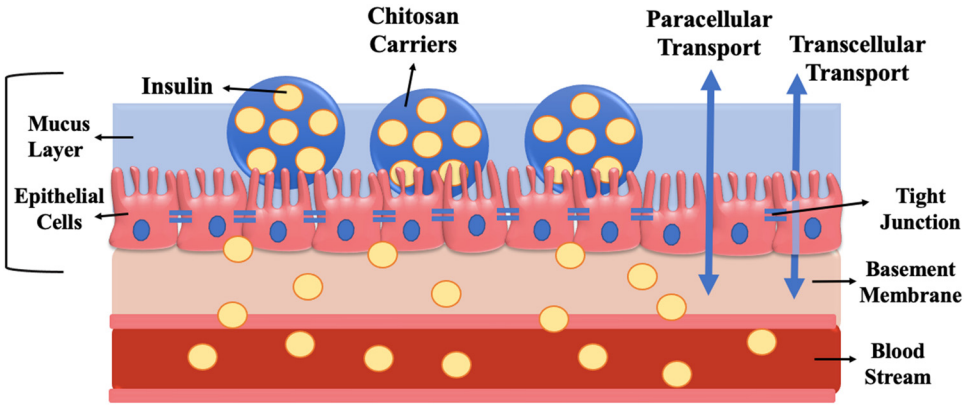

Figure 5: Illustrates the mucoadhesive property of chitosan and transferring the encapsulated insulin to the blood stream.

release capabilities of chitosan systems represent a leap forward in the precision and customization of OID strategies [103].

Quaternized derivatives of chitosan (QCs) have expanded the utility of chitosan for effective protein and peptide delivery in the neutral or weakly alkaline pH of the small intestine. Recently, chitosan nanoparticles (CS-NPs) were prepared using synthesized diethylmethyl chitosan (DEMC) and trimethyl chitosan (TMC) through the ionotropic gelation method or polyelectrolyte complex (PEC). Similarly, another study utilized newly synthesized tri-ethyl chitosan (TEC) and DMEC for NP preparation. In both investigations, insulin-loaded NPs were orally delivered to the colon. The encapsulation efficiency of insulin in positively charged CS-derivatized NPs ranged from approximately 70% (TMC, DEMC) to 90% (TEC, DMEC) when employing the PEC method. This high encapsulation efficiency is attributed to electrostatic interactions between the negatively charged acidic groups of insulin and the positively charged amino groups of CS derivatives. NPs derived from TEC and DMEC demonstrated sustained insulin release over 5 h, exhibiting minimal burst release. However, no significant deviation in the release profile was observed in phosphate-buffered saline (PBS) at different pH levels (6.8 and 7.4). Importantly, the derivatives exhibited higher insulin release compared to CS-NPs alone, as the quaternized derivatives showed enhanced solubility at neutral and alkaline pH levels [104–107].

9.3 Protection against enzymatic degradation

Enzymatic degradation in the GIT poses a significant threat to the stability of therapeutic proteins like insulin. Chitosan, acting as a protective barrier, shields insulin from enzymatic breakdown, ensuring that a substantial amount of the active drug reaches the bloodstream intact. This protective mechanism is crucial for maintaining the potency and bioavailability of insulin during its journey through the digestive system. The enzymatic protection offered by chitosan represents a strategic advantage in OID, addressing a fundamental challenge that has impeded the development of effective oral protein therapies [108].

Pai *et al.* have reported a method of preparing lipid NPs with enhanced physical stability *via* the incorporation of chitosan into the lipid NPs. The selection of the chitosan-lipid system depends on the drug's affinity to the matrix. Chitosan-lipid system and drug of similar affinities are selected to enhance the association efficiency of drug. The insulin is complexed with sodium docusate in formulation using the chitosan-monoolein system. The process of complexation removes or alleviates the charge density of insulin thereby increasing its affinity for lipophilic carrier. The formation of NPs comprising insulin-sodium docusate complex embedded in chitosan–lipid matrix is expected to increase the resistance of matrix and insulin against enzymatic degradation following its administration orally [109].

10 Modification of chitosan for colon targeting

By modifying its backbone of hydroxyl and amine groups, the properties of chitosan, such as mucoadhesion, stability, swelling degree, complexation ability, and solubility, can be modulated for specific applications. The main techniques commonly used in modifying chitosan include curing, blending or graft co-polymerization [110]. While in curing technique, complex methods such as electrochemical, ultraviolet radiation processing, and thermal methods are needed to convert polymers into a solidified mass by forming three-

dimensional bonds within a polymer network, blending is accomplished by mixing two or more polymers. In comparison, graft co-polymerization involves chitosan modification through covalent chemical bonding with grafting agents [111].

10.1 Physical modification

Chitosan modified by ionizing radiation, ultrasonic treatment, and mechanical grinding to design various shapes like gel particles, NPs, and sponge materials has shown great applicability. The most preferred technique endowing chitosan with desirable properties in practical application is polymer blending. Good mechanical and chemical properties have been reported when chitosan is blended with synthetic polymers such as polyvinyl chloride and polyvinyl alcohol. Moreover, physically crosslinked hydrogels are desired over chemically modified ones because of the uncomplicated formation of the physical polymeric network [112]. Different combinations of polysaccharides and polymers have been developed to improve colon-targeted drug delivery, as shown in Table 1.

10.2 Chemical modifications

Various modifications have been attempted to overcome absorption and enzymatic barriers of GIT by alteration of the polymer functional groups of chitosan. The type of chemical change is influenced mainly by the structural modifications involved in a given formulation, such as thiolation, quaternization, substitution, conjugation, and grafting [15].

10.2.1 Thiolation

Thiolation is a process where thiol groups are grafted to the backbone of the polymers using various types of thiolating agents such as thioglycolic acid, isopropyl-*S*-acetylthioacetimidate, 2-iminothiolane or 4-thiobutylamidine, cysteine, *N*-acetyl cysteine, and glutathione. The mucoadhesion property of chitosan can greatly be enhanced by means of thiolation through binding covalently to the mucosal layer as the thiol moiety reacts with the mucin-glycoprotein of the GIT by disulfide bond formation. Therefore, chitosan strongly binds to the mucosal layer and interferes with drug absorption while reducing drug diffusion [113,114]. In addition to its strong mucoadhesive ability, thiolated chitosan has permeability-boosting capabilities, block efflux pumps, and *in situ* gelling properties [115].

10.2.2 Quaternization

This method raises the pK_a of chitosan by adding a quaternary ammonium side chain, which changes the main amino group into a quaternary ammonium group. There are three ways to quaternate chitosan: direct quaternary ammonium substitution, epoxy derivative open loop, and N-alkylation [116]. The quaternization method can raise the solubility of chitosan at gastric pH when the hydrophobic functional groups are linked to the quaternized polymer or when adopting additional enteric coating [117,118].

10.2.3 Substitution

In this method, the functional groups are grafted to the hydroxyl group's oxygen or chitosan's primary amino nitrogen. The hydrophobicity of chitosan is increased when the hydrogen of amino groups is replaced with a long-chain acyl moiety, thus resulting in improved resistance to enzymatic actions and mucosal permeability [119].

10.2.4 Conjugation

In this approach, either chelating moieties are inserted into the system, or the extension of the pK_a of chitosan takes place. Polymer-ethylene glycol tetraacetic acid (EGTA) conjugation can result in calcium chelation in the vicinity of the oral insulin systems in the GIT. Calcium ions have a major role in enzymatic degradation in the GIT and the generation of the apical junctions along the mucosal layer, and these ions are required for the thermodynamic stability of proteolytic enzymes such as trypsin and chymotrypsin [98]. Therefore, chelating of Ca^{2+} ions can synergistically lower the enzymatic effects and enhance mucosal absorption. Similarly, conjugating glutamine with chitosan can change the pK_a of chitosan from 6.5 to 9.13, leading to positive charge retention, even in natural pH regions like the intestine or colon [15,120].

10.2.5 Grafting

Grafting is a method that copolymerizes a polymer into another polymer with negated effects on the original characteristics. Grafting was used in OID systems due to chitosan's inability to be ionically complexed with cationic or

non-ionic polymers such as polymethacrylic acid, which can enhance mucoadhesion [99]. Four grafting methods are primarily used in grafting chitosan: free radical-mediated grafting, electrochemical methods, radiation, and enzyme-catalyzed grafting. The grafting efficiency may differ according to the procedure parameters and method [121]. Nevertheless, grafting chitosan with polymethacrylic acid (incorporating *N*-vinyl pyrrolidone) increases hydrophilicity and poor mucin interaction with the formulation. This phenomenon can be explained by the fact that high levels of mucin adsorption have a detrimental effect on mucoadhesive delivery systems due to particle entrapment on the mucosal surface, which is eliminated by the dynamic nature of mucus [15,122].

The functional amine $-NH_2$ groups of chitosan provide a reaction site to facilitate binding with other active agents in developing different pharmaceutical applications, where they can react with citrates, phosphates, and sulfates, improving drug encapsulation efficiency and stability. For instance, the solubility of chitosan in intestinal media is improved by producing quaternized chitosan (*N*-trimethyl chitosan chloride). Trimethyl chitosan with a degree of quaternization in the range of 40–60% increases the intestinal permeability of hydrophilic macromolecular drugs. Moreover, formulating NPs with thiolated chitosan can further increase the mucoadhesiveness of chitosan [111].

10.2.6 Future perspectives

The future of OID through chitosan systems presents a promising frontier with the exploration of modified colon delivery strategies. Considering the challenges associated with insulin's transit through the GIT, including enzymatic degradation and poor bioavailability, focusing on colon-specific delivery mechanisms becomes crucial. Tailoring chitosan-based formulations for targeted insulin release in the colon could significantly enhance bioavailability, ensuring optimal therapeutic outcomes. Chitosan's unique properties, encompassing biocompatibility, mucoadhesion, and controlled release mechanisms, position it as a versatile platform. Specifically, in the context of modified colon delivery, the focus could be on optimizing chitosan carriers to ensure targeted insulin loading in the colon region. Chitosan modifications from different CS derivatives and CS complexes, such as N-sulphation, trimethyl CS, and diethylmethyl CS emerge as valuable carrier to enhance better insulin association efficiency and loading capacity in the intricate environment of the intestinal tract [109,118]. Looking forward, future perspectives might entail delving into advanced microencapsulation integration to fine-tune chitosan NPs, enabling breakthroughs in insulin bioavailability. Additionally, a shift towards personalized medicine approaches, tailoring chitosan carriers based on individual patient characteristics, could bring about more customized and effective strategies. The development of smart delivery systems responsive to specific GI conditions represent another intriguing avenue. Overall, this evolving landscape signifies a paradigm shift in diabetes care, offering a patient-friendly and efficient alternative to conventional insulin administration. Continuous innovation, interdisciplinary collaboration, and successful clinical translation will be pivotal in realizing the full potential of chitosan systems for OID in the modified colon context.

11 Preparation methods of chitosan NPs

Several types of NPs loaded with insulin for oral delivery by combining various materials with chitosan and chitosan modifications, including natural polymers, synthetic polymers, lipids, metals, and proteins have been developed (Table 2). Effective NP systems must be stable, non-toxic, biodegradable, non-thrombogenic, non-inflammatory, and nonimmunogenic and should escape their reticuloendothelial system. Furthermore, nanocarriers can be modified with particular ligands that target the receptors on the surface of epithelial cells to enhance peptides and protein uptake through oral administration.

Polyglutamic acid (PGA) is commonly applied in chitosan functionalization to enhance insulin uptake by amino acid transporters and calcium-sensing receptors present in the intestinal epithelium, as reported by Urimi *et al.* [143]. They have formulated chitosan-PGA insulin NPs with particle size of 210 ± 2.8 nm, zeta potential of 18.1 ± 0.14 mV, entrapment efficiency of 85.9% ± 0.28%, and sustained release profile at different pH conditions. Cellular uptake analysis showed a threefold higher uptake in the Caco-2 cell line, and the blood glucose study in diabetic animals reported low levels for almost 24 h. In another study, the preparation of insulin NPs by self-gelation technique involved natural polymers like chitosan and snail mucin [134]. The resulting NPs were smooth with an average particle size of 504.1 nm, a positive surface charge at 31.2 mV, low polydispersity index (PDI), encapsulation efficiency of 92.5%, and controlled release for 8 h. The *in vivo* study on diabetic rats demonstrated that oral insulin NPs lowered blood glucose levels more efficiently than the free oral insulin solution.

Chitosan NPs can be prepared using various methods such as coacervation, emulsion cross-linking, ionic gelation, reverse

Table 2: Formulations of insulin chitosan NPs

Formulation	Method of preparation	Chitosan concentration (% w/v)	Size (mean value ± SD) (nm)	Zeta potential (mV)		Encapsulation efficiency (%)	Loading capacity (%)	Release profile	Ref.
Chitosan-coated mPEG-b-PLGA NPs	Double-emulsion (water-in-oil-in-water) solvent evaporation method	0.5		224.4 ± 13.8	+13.7 ± 1.6	55.2 ± 7.0	4.9 ± 0.7	pH 1.2 13.91% pH 6.8 38% 4 h	[155]
Polyurethane–alginate/chitosan core-shell NPs	Polyelectrolyte complexation method	1		156.12	38.5	90	12	pH 1.2 15.77 2 h pH 6.8 50% 10 h pH7.4 100% 20 h	[156]
pH-responsive carboxymethylated iota-carrageenan/chitosan NPs	Polyelectrolyte complexation method	0.1		613 ± 41	52.5 ± 0.5	86.9 ± 2.6	10.7 ± 0.6	pH 1.2: 5% 2 h pH: 6.8: 87% 12 h	[157]
Insulin-loaded chitosan-NPs	Complex coacervation	0.5		534 ± 24	14.57 ± 1.1	79.96 ± 3.96	—	pH 2 13% 10 h pH 6.8 88% 10 h	[9]
Insulin-loaded NPs	Self-gelation method	2		479.6	22.1–31.2	88.6	—	pH 1.2: 7% 4 h pH 7.4: 80 % 10 h	[134]
Insulin-loaded trimethyl chitosan-fucoidan NPs	Simple PEC	0.2		256.7 ± 4.9	26.5 ± 1.1	56.4 ± 4.3	8.6 ± 2.2	SGF 38.3 SBF 75.4	[158]
Chitosan/lecithin liposomal nanovesicles	PECs associated with lecithin liposomes	—		105 ± 17	−30	20	—	PBS 6.8: 80% after 20 min	[159]
Insulin loaded chitosan-TPP NPs	Flash nano-complexation using a multi-inlet vortex mixer	—		46.2 ± 2.7	9.4 ± 1.2	91.0 ± 1.7	27.5 ± 0.4	pH 2.5: 55% pH 6.6: 10% pH 7:23% after 1 h	[131]
Insulin-loaded chitosan nanoparticles (INS-CS-NPs)	Ionic gelation	0.3		356.5 ± 43.4	46.5	78.3%	—	pH 2.5: 40% pH 6.8: 50% 2 h	[160]
Chitosan-alginate (CS/ALG) NPs encapsulated in insulin	Polyelectrolyte complexation	—		216	+3.89	78.3	—	pH 1.2: 26.7% 2 h pH 6.8: 79% 12 h pH 7.4: 84% 12 h	[161]

microemulsion, and spray-drying methods. The choice of preparation method depends on various factors, such as the intended application and desired properties of the chitosan NPs [144,145]. Several formulation and process parameters should be optimized to achieve superior characteristics of the NPs that suit the intended objectives.

11.1 Coacervation

Coacervation is a separation process induced by a change in the media environment, such as ionic strength, temperature, solubility, or pH, under a controlled condition. NPs are formed due to water deficit or dehydration mechanism after liquid desolvation or salt's addition into the reaction medium (Figure 6) [146]. For polysaccharides to develop coacervate droplets, chitosan solution must first be blown into an alkali solution such as NaOH, methanol, or ethanedi-amine using a compressed air nozzle. Then, the formed particles are centrifuged, followed by successive washing with cold and hot water before introducing the crosslinking agent for further hardening [32].

Oral insulin chitosan-Dz13Scr NPs were fabricated by means of a complex coacervation method [147]. The produced NPs showed enhanced stability in the presence of stomach acid, and in the presence of alkaline conditions, a biphasic release behavior of the drug was observed, with an initial burst release followed by a more regulated release. In addition, the study reported that the NPs formulation was shown a glucose uptake into in C2C12 cell line. The NPs are prepared at low temperatures using aqueous solutions; hence coacervation method is safe and appropriate for temperature-sensitive drugs, including proteins. However, some drawbacks are associated with this method, such as poor encapsulation efficiency and system instabilities [148].

11.2 Emulsion crosslinking

It is one of the most straightforward techniques that can encapsulate liquid and solid materials to produce various particle sizes [149]. It involves crosslinking the functional groups of the coating material with the crosslinking agent. This approach entails water formation in oil emulsion (W/O) through emulsifying the coating solution in the oil phase, which can be stabilized using appropriate surfactants. Once a stable emulsion is established, a crosslinking agent such as TPP is introduced to solidify the emulsion droplets (Figure 7) [32]. This method is simple to scale up and suitable to encapsulate hydrophobic drugs, while the drawbacks include high-shear and incorporation of organic solvents and surfactants [6]. In these crosslinked emulsions, a sustained release of insulin can be achieved by varying the ratio of polymer to surfactants. For example, it was reported that a microemulsion of insulin administrated orally showed slower rate of glucose depletion but a more sustained release of insulin compared to solution and subcutaneous formulations. In

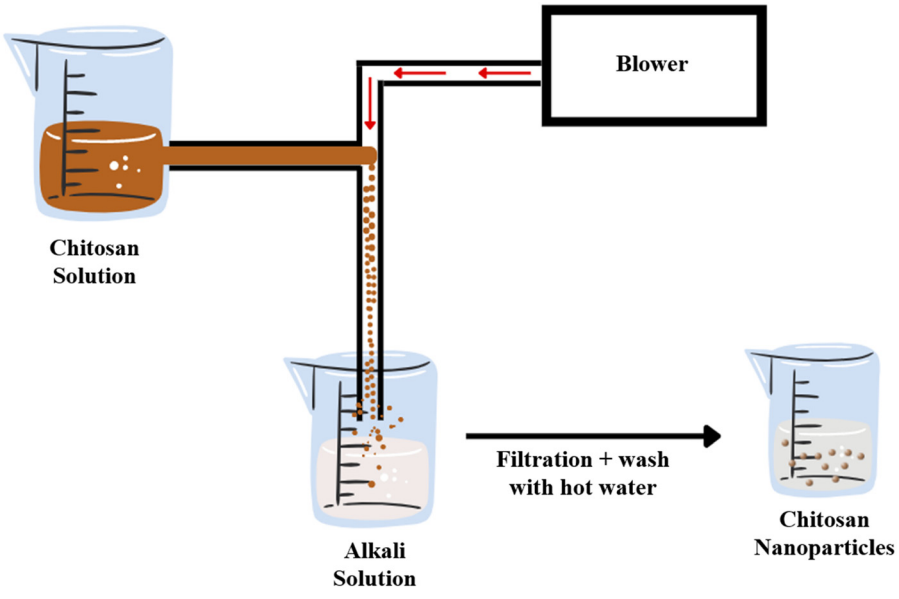

Figure 6: Schematic presentation of the coacervation separation process.

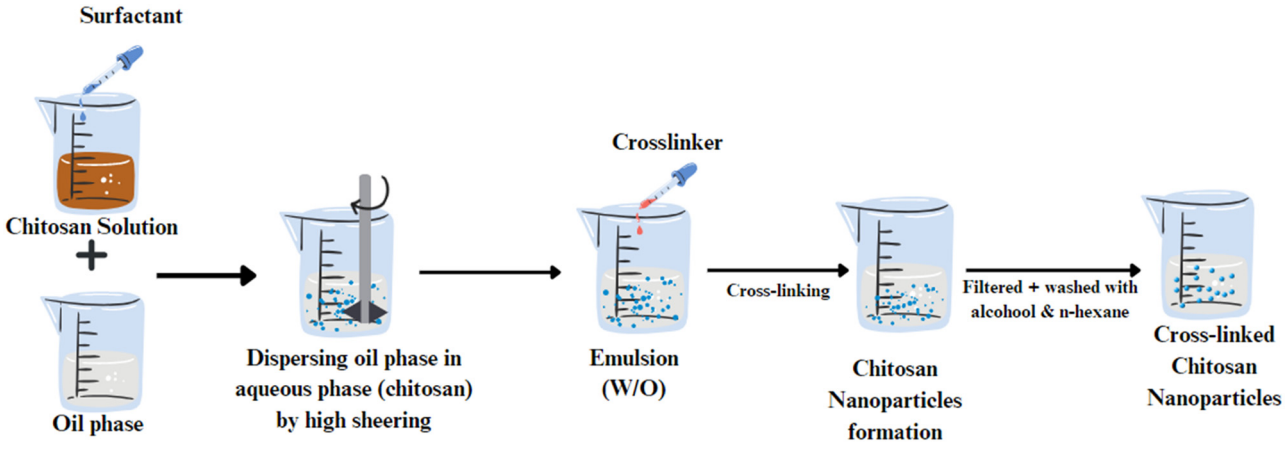

Figure 7: Schematic presentation of the emulsion crosslinking process.

another study, it was reported that insulin delivery was controlled using a various ratio of mucin to Tween® 80 in oil/water microemulsions [150].

Fonte and his team 2011 fabricated a type of solid lipid nanoparticle (SLN) containing insulin and a chitosan coating. To prepare these SLNs, they used a technique that involved creating a double emulsion of water and oil and then applying chitosan to the surface of the SLNs. The optimized formulation showed a particle size of around 450 nm with a positively charged surface. A greater extent of NPs permeation was reported after adding chitosan. The hypoglycemic effect of diabetic rats was observed for 24 h with relative pharmacological bioavailability of around 17% (oral absorption of insulin is improved by SLNs coated with chitosan) [151].

11.3 Ionic gelation

The approach of this method is based on electrostatic interaction between different charged polymers under mechanical stirring. In this method, various chitosan carriers including beads, microbeads, MPs, and NPs can be produced. This is achieved by adding a crosslinker solution, such as TPP, dropwise to a chitosan solution under constant high stirring. This process causes chitosan to undergo a gel ionization process by the electrostatic interaction between the polyanion groups of the TPP and the cationic amino groups of chitosan, resulting in NPs formation (Figure 8). Then, the prepared particles can be harvested by precipitation centrifugation techniques.

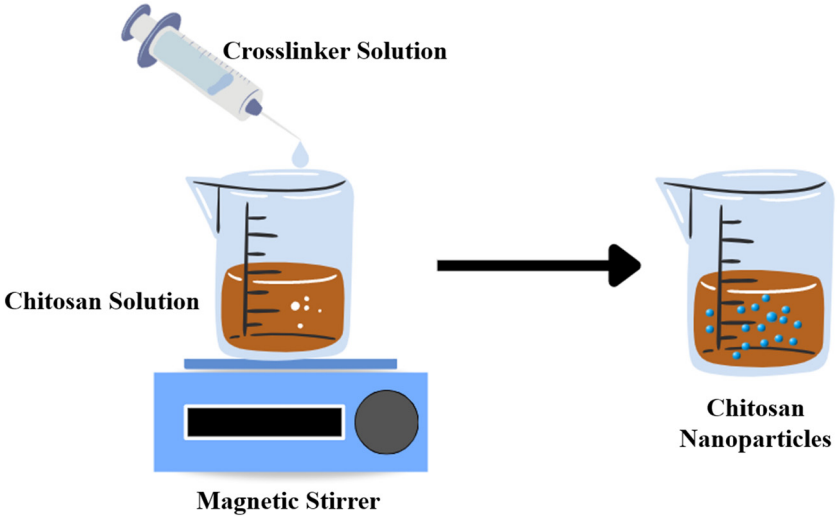

Figure 8: Schematic presentation of the ionic gelation process.

In the ionic crosslinking arrangement, the main types of interactions are H-links and T-links. An H-link occurs when O^- and NH^{3+} molecules interact in the same plane. Conversely, a T-link happens when a non-binding oxygen atom and NH^{3+} molecules located in different planes interact with each other [152]. The polyanion (negative charge), including sulfate salts (Mg, Na, or hexadecyl-, lauryl-, octyl-, cetostearyl-, chloride salts (Cu, Zn, Ba, Ca, Mg, and Co), polyphosphate salts (pyro-, tri-, tetra-, octal-, and hexameter-), ferricyanide salts, and ferrocyanide were used as a crosslinker for the synthesis of chitosan NPs. Chitosan polymer and TPP can be seen as a very well-known pair of counter ions in this method [152]. Ionic gelation consists of a simple preparation procedure that can be carried out under mild conditions, using cheap equipment and free of organic solvents. However, the disadvantage of this method is the high PDI of the prepared NPs [6]. The synthesis of chitosan NPs is influenced by chitosan concentration, crosslinker concentration, chitosan molecular weight, CS/TPP ratio, drug concentration, pH of the preparation medium, stirring rate, and crosslinking time [152].

Mahdizadeh Barzoki *et al.* have developed and optimized a novel oral insulin NP delivery system using thiolated *N*-dimethyl ethyl chitosan (DMEC-Cys) conjugate by means of an ionic gelation method [153]. The particle size measured 148 nm with a PDI of 0.26, a zeta potential of 15.5 mV, and an encapsulation efficiency of 97.56%. The release of insulin amounted to approximately 13.2% and 11.42% in pH 6.8 and 7.4 media, respectively. El Leithy *et al.* have designed a system for controlling glucose levels in rats for an extended time using insulin folate-chitosan NPs [154]. The NPs were positively charged and had a particle size of 288 ± 5.11 nm, with an entrapment efficiency of 80%. In these NPs, the stability of insulin when exposed to GI enzymes was enhanced, leading to a release of less than 10% at pH 1.2 and a release of 38.92 ± 4.52% over 8 hours at pH 7.4.

In another study, Xu *et al.* have developed a new system for delivering insulin orally, called NiM (NPs in MPs), which addresses the challenges posed by GI barriers [69]. It comprises a three-dimensional system of biocompatible chitosan, alginate, and casein. Initially, alginate/chitosan NPs were prepared by ionic gelation and complexation method, followed by coating with casein to form NiM, which helps to maintain insulin stability and physiological activity in the stomach. The NiM was stable in GI with an entrapment efficiency of 51.1%, while the in vitro insulin release was 13.5% in the simulated gastric fluid after 2 h followed by a slow release of 57.4% in simulated intestinal fluid over 10 h [69].

12 Preparation methods of chitosan MPs

MPs have the potential to provide an extensive reservoir for drug-coated NPs to exhibit a stimulus release at the target site. In the context of targeting the colon region, an oral system employing NiM can effectively safeguard the encapsulated drug in NPs from chemical and enzymatic degradation effects during GI transit while also reducing or eliminating the burst drug release in the upper GI and maximize the release of the encapsulant by intestinal or colon's stimuli. For example, a recent study reported Green Fluorescent Protein NPs encapsulated in alginate/chitosan MPs for colon-specific release and stomach transit [10,60]. Table 3 represents examples of insulin chitosan MP formulations.

In 2020, a group of researchers applied polysaccharides to fabricate an effective system for oral delivery consisting of hydrogel MPs to control the release and enhance paracellular and transcellular insulin absorption. In their study, carboxymethyl chitosan hydrogels were grafted by carboxymethyl βcyclodextrin (CMCD-g-CMCs) using carbodiimide as a crosslinker. The insulin released in the acidic medium (SGF, pH = 1.2) was just 8% after 2 h. After changing the release medium to pH = 6.8, the rate of insulin release was significantly raised to around 55% after 2 h, while it reached 70% in the medium with pH = 7.4 after 4 h [162].

Kim *et al.*, have prepared chitosan insulin microspheres using ionic crosslinking with phytic acid (PA). The microspheres showed a good encapsulation efficiency (97.1%), with a diameter of 663.3 μm, and the amount of insulin released in a gastric digestion medium was 67.0% after 2 h. The pharmacological bioavailability study was at 10.6%, significantly decreasing blood glucose levels [163].

12.1 Electrospray

In the electrospray process, a high voltage is applied to a solution containing the material of interest, typically a polymer or a drug. As chitosan solution is ejected through a stainless steel needle or multiple needles, the electrostatic forces cause the solution to form cone-shaped droplets, known as Taylor cones (Figure 9). As the voltage increases, the droplet's surface tension decreases until it reaches a critical point, and the droplet is ejected from the needle as a highly charged jet toward a collector. The extruded particles away from the needle are mono-

Table 3: Formulations of insulin chitosan MPs

Formulation	Method of preparation	Chitosan concentration (% w/v)	Size (mean value ± SD) (μm)	Encapsulation efficiency (%)	Loading capacity (%)	Release profile	Ref.
PA-crosslinked chitosan microsphere	Ionic crosslinking with PA	—	663 ± 40	97.1	7.6 ± 0.3	pH 1.2: 33% 2 h pH 6.8: 70% 4 h	[163]
Insulin-loaded chitosan microspheres along with polyvinyl alcohol	Spray drying	1.0	1.116 ± 0.507	—	4.3 ± 02	pH 6.5: 30% 2 h	[176]
Insulin-loaded water-soluble chitosan-MPs	Polyelectrolyte complexation	—	0.292	48.28 ± 0.90	9.52 ± 1.34	pH 1.2: 50% 2 h pH 7.4: 50% 4 h	[177]
Insulin-loaded *N*-trimethyl chitosan MPs	Supercritical fluid-assisted atomization	—	1.34	9.24	9.24 ± 4.7	—	[178]
Insulin-loaded chitosan MPs	multiple emulsion technique	2.0	37.5 ± 0.3	80.1 ± 0.1	28.0 ± 0.0	—	[179]
Chitosan-pectin Np and MP	Electrostatic self-assembly	0.5	0.240–1.900	62.0%		pH 1.2: 13% 2 h pH 6.8: 80% 2 h	[128]

positively charged and tend to homogenously distribute in the collecting solution [164]. The basic experimental setup mainly consists of a syringe pump, a metal nozzle connected to a high-voltage power source, and a collector. A transparent electrospray chamber is recommended to determine the electric field focus, temperature, humidity, and atmospheric pressure. This method is influenced mainly by the solution flow rate, applied electric voltage, and the solution properties such as viscosity, surface tension, and electrical conductivity [165]. Electrospray is a simple and low-cost technique that can control the particle size (usually this technique can produce particles with their sizes ranging from nanometers to several micrometers depending on the flow rate and the voltage applied) and yield using low amounts of solvents [166,167]. Even though it is employed to produce a variety of sizes based on the experimental set-up, MP preparation is more efficient concerning yield and time compared to smaller sizes since the flow rate and particle size are directly proportional [168].

12.2 Membrane emulsification

This process involves injecting a dispersed phase (aqueous) into a continuous phase (immiscible liquid or pre-emulsified mixture) through a microporous membrane that leads to the production of droplets at the continuous/membrane phase interface [169]. Chitosan solution is used as a dispersed phase, while an oil-soluble emulsifier is a constant phase. Nitrogen gas is used to press the chitosan solution through the pores of a membrane wall into the oil phase, forming a W/O emulsion with homogeneous droplet sizes with the presence of an emulsifier [170]. Solidifying the droplets can be achieved using a conventional stepwise crosslinking method with a crosslinking agent such as TPP or glutaraldehyde, as shown in Figure 10 [171]. This method easily controls the size of chitosan microspheres with a good PDI. The interfacial tension between the membrane and the dispersed phase must be high to form microspheres of uniform size [31,32].

In a previous study, researchers used a membrane emulsification technique and Ca^{2+} ion and polymer (chitosan) solidification to prepare alginate-chitosan microspheres. These microspheres were then used to load insulin, where the effect of various loading methods on loading efficiency and immunological activity was examined. The findings showed that insulin-loaded microspheres prepared using the chitosan solidification process had improved loading efficiency (56.7%) and remarkable activity maintenance of insulin (99.4%). The release profile showed that 32% of the loaded insulin

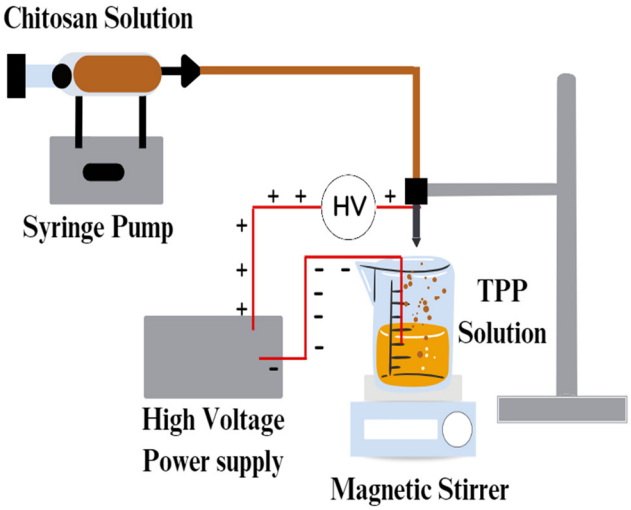

Figure 9: Schematic presentation of electrospray process.

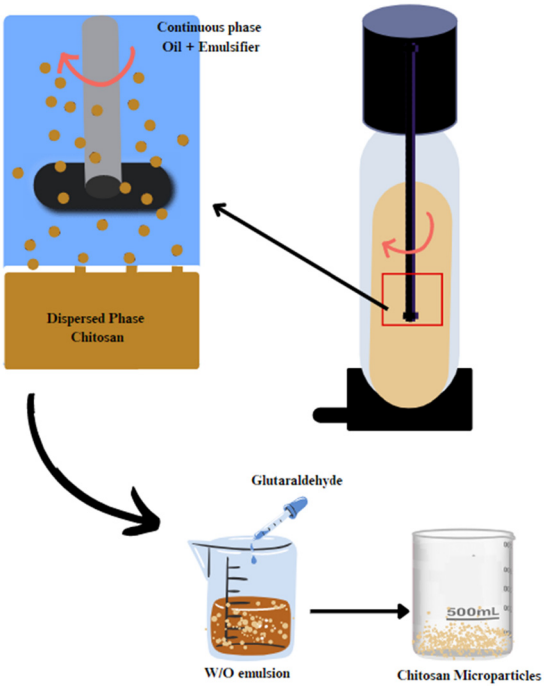

Figure 10: Schematic representation of the membrane emulsification process.

was released into simulated GI media. In addition, a continuous insulin release was recorded in pH conditions similar to the blood environment for 14 days. An *in vivo* study using diabetic rats showed a stable reduction in blood glucose levels for approximately 60 h [172].

Another study described an innovative approach for delivering insulin orally using hollow quaternized chitosan microspheres that uses the SPG membrane emulsification process and glutaraldehyde cross-linking procedure. These microspheres had uniform size, autofluorescence, and structural properties that helped maintain insulin bioactivity. In addition, the particle size was around 7.5 µm, which was small enough to be absorbed by the GIT and target the reticuloendothelial system after oral administration was achieved. Unlike conventional methods, the SPG membrane technique produced microspheres with a narrow size range and low coefficient of variation [173].

12.3 Microfluidic technology

Microfluidic technology is a device used to fabricate uniform emulsion droplets. This technique involves a delicate balance between various forces acting on the fluid flow, such as the periodic breakup of the dispersed phase into the continuous phase, mainly driven by viscous force, inertial forces, buoyancy, and interfacial forces. Conventional methods for forming emulsions typically involve droplet breakup using high-pressure homogenization and/or ultrasonication and rotary agitation, resulting in highly polydisperse droplet sizes due to the nonuniform shear stresses applied [33,34]. The preparation of MPs using the microfluidic method involves two steps, the formation of emulsion, where the polymeric or monomer fluid emulsifies to form the emulsion droplets in the channels, and the polymerization or curing of the droplets *in situ* to obtain microspheres (Figure 11). The curing methods generally involve freezing, solvent evaporation, and polymerization [165]. Compared to other methods, the microfluidic method produces MPs with a unified size and narrow size distribution, good process repeatability, and a more stable yield. However, microspheres produced by microfluidics are compact, with a relatively small specific surface area and slow adsorption rate, resulting in a low adsorption capacity [168]. Moreover, other microfluidic limitations include low flow rate, low yield, and clogging of channels due to the adhesion of small particles [169].

12.4 Reverse micellar method

Reverse micelles are droplets of an aqueous phase stabilized through a surface-active agent (surfactant) in an organic phase. They are formed when organic and aqueous phases are mixed in the presence of a surfactant (Figure 12). The efficiency of the approach and the stability of the prepared reverse micelles mainly depends on the processing parameters, the physicochemical properties, and the ratios of the components and surfactants used [170]. Employing

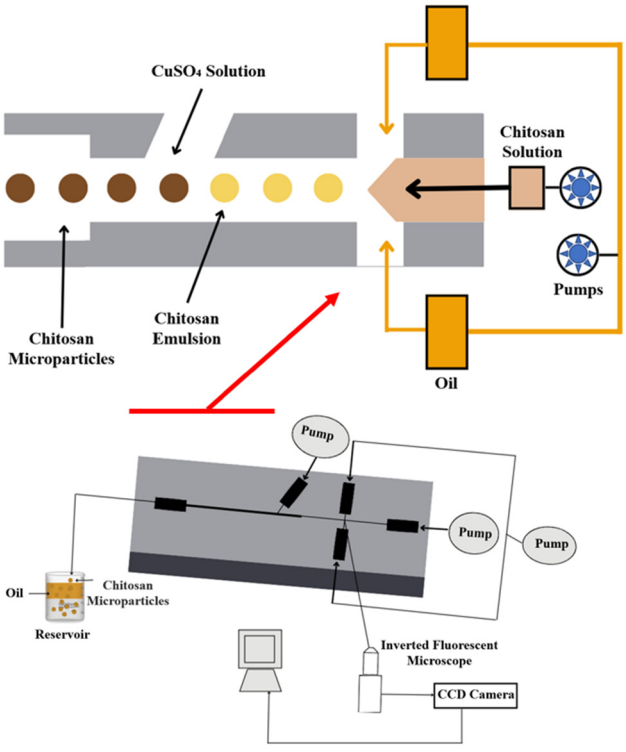

Figure 11: Schematic representation of the microfluidic method.

chitosan with low molecular weight is superior for controlling MP size and size distribution. However, the main limitation of this method is the usage of organic solvents and the collection of the MPs [171].

12.5 Sieving

In the sieving method, a chitosan solution is pre-crosslinked and then pushed under a certain pressure to pass through a sieve with a suitable mesh size to form chitosan MPs (Figure 13). The homogeneous pore size of the sieve produces MPs with a very narrow size distribution. To remove any unreacted excess glutaraldehyde (crosslinker), particles need to be washed with NaOH and dried in an oven at 40°C overnight [32]. This method is characterized by easy scalability and preparation, but the MPs produced possess irregular shapes [31].

12.6 Spray drying

Spray drying is a common method for producing granules, agglomerates, or even powder from a mixture of excipients and APIs. The method involves spraying suspensions or solutions into a closed chamber through a nozzle. The

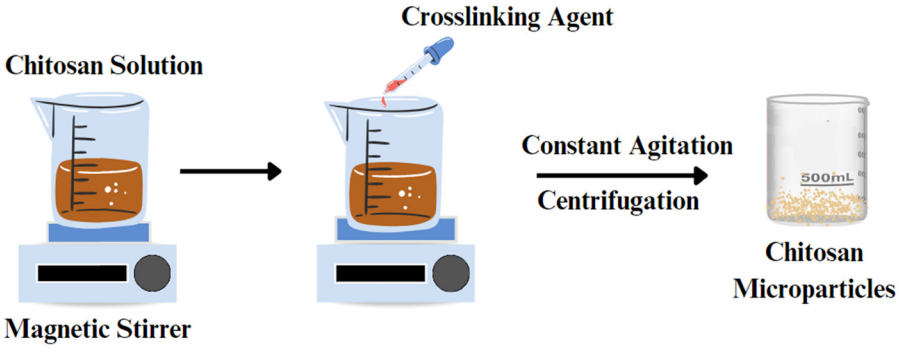

Figure 12: Schematic representation of the reverse micellar method.

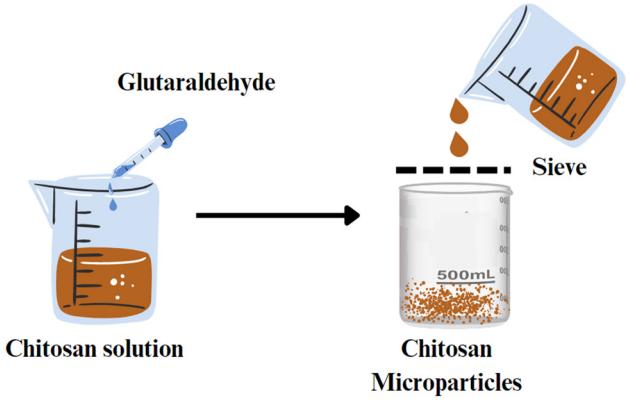

Figure 13: Schematic representation of the sieving method.

resulting atomized droplets undergo rapid evaporation drying using a hot air flow at constant pressure and temperature. The final dried particles are separated from the drying gas and collected in a collection vessel (Figure 14) [32]. According to Shehata and Ibrahima, this method can produce spherical-shaped spray-dried particles with low values of the angle of repose, which leads to good flowability during capsule filling or tablet compression. However, a loss in yield was reported due to powder sticking on the scraper and electrode in the cyclone and the collection chamber [174]. Insulin-chitosan MPs- or NPs can be produced through this method by optimizing the processing parameters such as nozzle size, inlet and outlet temperature, feed flow rate, the concentration of the insulin-chitosan solution, air flow rate, and atomizer [175]. Table 3 summarizes chitosan MPs prepared using different methods and techniques for OID.

13 Preparation methods of chitosan beads

Chitosan hydrogel beads are three-dimensional networks of chitosan chains crosslinked with different chemical agents or by physical means. These beads have drawn much interest in drug delivery because of their unique qualities, including biocompatibility, biodegradability, and the capacity to expand and release medications under controlled conditions [180]. Chitosan hydrogel beads can absorb much water and swell in aqueous environments. Several parameters, such as temperature, pH, and degree of cross-linking, influence the swelling behavior of these beads. The swelling capacity of chitosan hydrogel beads can be modulated by altering the degree of crosslinking, and it is generally inversely proportional to the degree of cross-linking.

Furthermore, the swelling behavior of chitosan hydrogel beads depends on the surrounding media's pH, where the beads swell more at higher pH values [181]. Chitosan beads have been widely used in drug delivery applications due to their ability to control drug release. The drug release behavior from chitosan hydrogel beads is determined by several factors, including the degree of crosslinking, size and shape of the beads, drug solubility, and pH of the release medium [182]. Several parameters of the chitosan hydrogel bead preparation process can affect the physicochemical properties of the beads, including chitosan concentration, crosslinking agent concentration, pH of the reaction medium, and reaction time. The choice of these parameters can affect the degree of crosslinking, the size and morphology of the beads, and their swelling and drug-release properties. Therefore, optimizing these parameters is essential for obtaining chitosan hydrogel beads with desirable properties for specific applications [183].

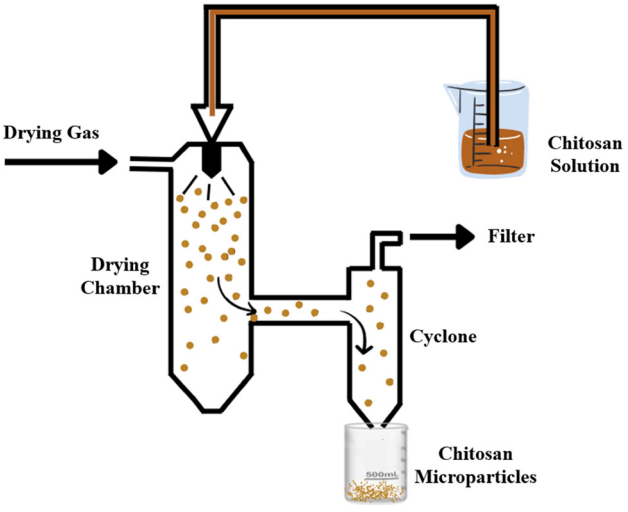

Figure 14: Schematic representation of spray drying method.

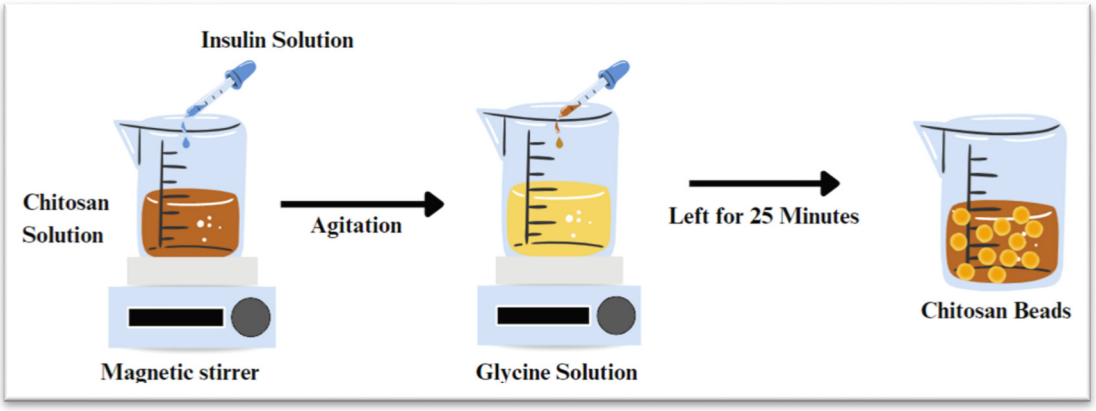

Figure 15: Schematic representation of ionic gelation method.

13.1 Ionic gelation for beads preparation

Ionic gelation is commonly used to form beads where chitosan is dissolved in an acidic solution to form a polycationic solution. Then, the chitosan solution is dropped into an alkaline coagulating solvent that instantaneously forms spherical beads (Figure 15). However, chitosan beads' mechanical strength and acid resistance properties usually require optimization to meet specific purposes. In order to enhance the mechanical stability and adsorption capacity of the beads, cross-linking agents like glutaraldehyde, glycol diglycidyl, and epichlorohydrin can be used [184].

In a study conducted by Kofuji *et al.* insulin chitosan gel beads using the ionic gelation method were reported by dropping insulin suspension into chitosan solution with agitation. Then, the mixed solution was dropped slowly into an aqueous glycine solution and left under gentle stirring for 25 min at room temperature, and the beads were then formed spontaneously [185]. Ionic gelation in producing beads or microbeads is considered a preferred method due to its lower cost, flexibility in producing particles with various ranges of size, high to medium drug encapsulation efficiency, good stability, and controlled drug release. However, it shows limitations in the concentration range of the polymer [186].

13.2 Simultaneous crosslinking

Simultaneous crosslinking is a one-step method for preparing chitosan beads by covalent cross-linking and precipitation or solubilization in an alkaline aqueous medium (Figure 16) [187]. This method can prepare chitosan beads or hydrogels due to the cross-linked hydrophilic polymer nature and can absorb large amounts of biological fluids or water. Crosslinking between polymer chains provides benefits such as increased elasticity, polymer insolubility, reduced viscosity, increased strength, and reduced melting point [187,188]. However, the main concern with this method is its lower reactivity due to many functional groups being buried in the polymer matrix [189]. A study conducted by Barreiro-Iglesias *et al.* found that chitosan beads can be prepared by mixing the solutions of chitosan and glutaraldehyde (as a condensing agent) in purified water for a few seconds and then adding them dropwise to NaOH solution at 20 or 37°C [187].

14 Chitosan as coating material for colon insulin delivery

Chitosan has been widely used as a surface coating and particle-forming polymer. This coating was widely employed with many types of NPs like lipid NPs, metal-based NPs, and polymeric NPs. It is used for mucoadhesive enhancement, tissue penetration property, drug release, bioavailability improvement of drug-loaded, and stability control of chitosan-coated drugs [190]. In some studies, chitosan and chitosan derivatives were employed as coating materials to protect insulin NPs by increasing the residence time of NPs in intestinal mucosa and avoiding premature insulin release in the gastric medium [191]. Other than being covalently attached to chitosan, the drugs are also dispersed in the chitosan matrix, released by controlled and slow diffusion through/from the chitosan. The enzymatic action of colonic microflora enables chitosan to release the entrapped drug, specifically within the colon, like, β-glycosidase while retaining the matrix integrity in the upper GIT [192].

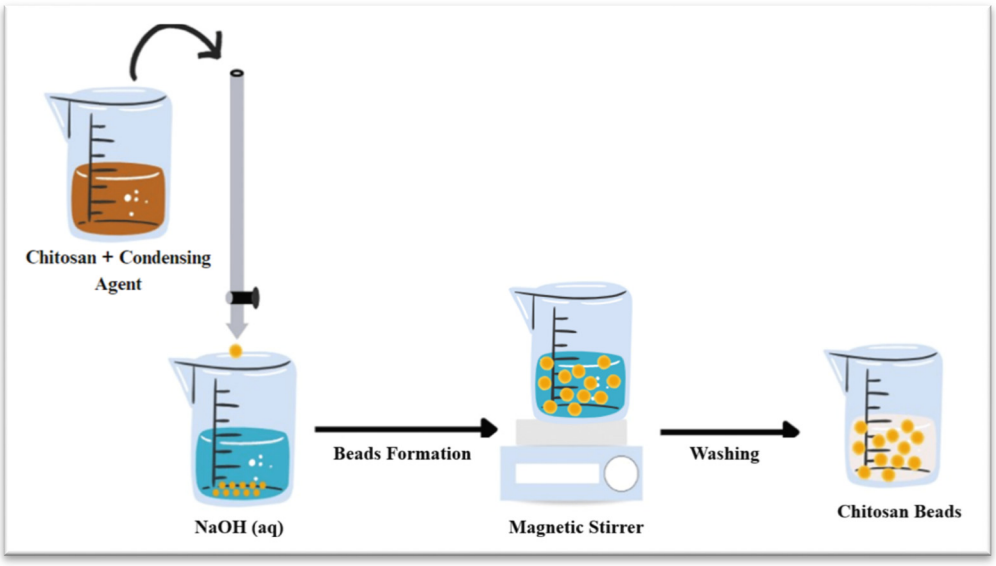

Figure 16: Schematic representation of simultaneous crosslinking method.

15 Future perspectives of chitosan-based formulations

The challenges associated with OID have provided a significant opportunity for research initiatives. Addressing issues such as the acidic environment of the stomach, enzymatic degradation, and the mucus barrier in the GIT has been a substantial point of investigation. Chitosan, as a polysaccharide polymer, exhibits notable characteristics including biocompatibility, biodegradability, and low toxicity. These attributes make chitosan as a promising material for the development of formulations aimed at overcoming challenges linked to OID. As research advances, the exploration of chitosan-based formulations continues to hold potential for transforming oral insulin drug delivery systems. Thus, chitosan-based formulations emerge as promising approaches for OID.

Insulin has been formulated for oral delivery in various chitosan-based formulations utilizing microencapsulation approaches, including NPs, MPs, and relatively large matrices such as beads. Microencapsulation, employing chitosan as a wall material, has resulted in a promising OID and colon-targeting system. Future perspectives of chitosan-based formulations for OID may focus on enhancing its bioavailability and increasing protection in the GIT. Achieving these objectives could involve physical and chemical modifications of the wall material, combining chitosan with other functional polymers, or refining the fabrication methods. Furthermore, formulating complex systems, such as microencapsulating chitosan-insulin NPs/MPs within larger delivery systems, holds potential for overcoming challenges related to insulin bioavailability and achieving a successful controlled release profile. This approach requires further exploration through biological studies, followed by subsequent clinical trials, to comprehensively assess its efficacy and safety in practical applications.

16 Conclusion

The current insulin administration route has several limitations, including the need for frequent injections, which can lead to poor patient compliance. OID represents an attractive alternative to current methods, but various barriers limit its success. Chitosan-based polymers are a promising carrier matrix for OID due to biocompatibility, biodegradability, and low toxicity. Physical and chemical modifications of chitosan can further improve its efficacy in insulin delivery by increasing its solubility, mucosal permeability, and improving insulin protection in the GIT. Modification techniques such as thiolation, substitution, conjugation, and grafting can further enhance the ability of chitosan to protect insulin and promote its release. Various chitosan carriers, such as NPs, MPs, and beads, have been developed to enhance OID, and many of them exhibited promising results for oral colon delivery. These carriers have shown great potential in overcoming the challenges of oral insulin delivery, including acidity, enzymatic degradation, and mucus barrier and attempted to improve colon insulin absorption. Numerous preparation methods,

including coacervation, emulsion crosslinking, ionic gelation, electrospray, and spray drying, can produce all sorts of chitosan carriers for drug delivery with enhanced physicochemical properties. While there are still challenges in improving oral insulin bioavailability with a controlled insulin release profile, chitosan-based carriers have shown promising results in preclinical studies and represent a promising avenue for developing an OID system.

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