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Chapter

Chitosan-Based Oral Drug Delivery System for Peptide, Protein and Vaccine Delivery

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Abstract

Oral delivery is the most common and preferred route of drug administration due to its convenience and ease of administration. However, various factors such as poor solubility, low dissolution rate, stability, and bioavailability of many drugs remain an ongoing challenge in achieving desired therapeutic levels. The delivery of drugs must overcome various obstacles, including the acidic gastric environment, the presence of the intestinal efflux and influx transporters and the continuous secretion of mucus that protects the gastrointestinal tract (GIT). As the number and chemical diversity of drugs has increased, various strategies are required to develop orally active therapeutics. One of the approaches is to use chitosan as a carrier for oral delivery of peptides, proteins as well as vaccines delivery. Chitosan, a non-toxic N-deacetylated derivative of chitin appears to be under intensive progress during the last years towards the development of safe and efficient chitosan-based drug delivery systems. This polymer has been recognised as a versatile biomaterial because of its biodegradability, biocompatibility, and non-toxicity. This chapter reviews the physicochemical characteristics of chitosan and the strategies that have been successfully applied to improve oral proteins, peptides, and vaccines bioavailability, primarily through various formulation strategies.

Keywords: chitin, chitosan, bioavailability, oral delivery, peptides, vaccines

1. Introduction

The extent of drug bioavailability has been shown to be influenced by the route of drug administration. Oral drug route needs travelling through the continuous passageway of the GIT, which makes them susceptible to the harsh environment of GIT. Drugs intended for administration via this route can be formulated in a variety of dosage forms, such as tablets, capsules, solutions, and powders.

Due to its high patient compliance and ease of administration, the oral route of administration is preferred among other routes. Self-administration is possible with great compliance and reduced risk in developing systemic side effects, which is the major concern in the parenteral route [1]. Despite that, the oral delivery system approaches for certain drugs are challenging, especially the delivery of peptide drugs and vaccines [2].

The normal physiological functions of GIT are to digest food and to interfere with pathogen entry. These functions need to be considered as peptide drugs and vaccines tend to be digested together with food in the presence of digestive

enzymes. The highly acidic pH in the stomach and the presence of proteolytic enzymes such as protease and pepsin can cause protein degradation [1].

Furthermore, they will have difficulties in permeating the physical barrier of the mucus lining, which prevents pathogenic substances from penetrating the cell [3]. Owing to these challenges, protein and peptide drugs are suitable to be administered via parenteral routes such as intravenous or subcutaneous injection [4]. However, these routes require frequent administration with long-term use which will develop patient incompliance to medication [4]. In such a manner, the approach to improve the oral delivery of peptide drugs and vaccines by using suitable polymers are needed to enhance drug effectiveness and patient compliance.

Chitin is the second most abundant polysaccharide present in nature. However, it has more applications when converted to chitosan by partial deacetylation under alkaline conditions [5]. Chitosan is a positively charged polymer that can improve the bioavailability of the oral drug delivery system. It has been used to improve the formulation of peptide drugs, resulting in enhanced cell permeability, which allows an adequate therapeutic concentration of drugs into the systemic circulation [6].

For protein and peptide therapeutics, factors such as poor permeability, luminal, brush border, cytosolic metabolism, and hepatic clearance mechanisms result in their poor bioavailability from oral and non-oral mucosal routes [7]. Oral vaccination is prone to reduce the adequacy of vaccine to be recognised by the immune system due to the presence of gut microbiota and intestinal barrier. Peptide drugs and vaccines can be protected from the degradative barrier of the GIT by encapsulating the drugs into the polymeric chitosan as potential carrier material. The development of nanotechnology, such as nanoparticle systems to transport peptide drugs through the epithelial membrane has been established [6, 8]. Besides, the modification of chitosan is needed to exert its function as a polymer and to protect the drug from enzymatic degradation [9].

This work reviews the physicochemical properties and numerous applications of chitosan, describes its release mechanisms, challenges in oral peptides and vaccines delivery, and strategies to overcome these barriers to improve oral peptides and vaccines bioavailability.

1.1 Chitosan

Chitosan is a strong base with linear polysaccharides consisting of D-glucosamine, which contains amino groups [10]. The hydrolysis of chitin will produce chitosan through alkaline hydrolysis or N-deacetylation (**Figure 1**). Due to protonable amino groups presence in chitosan, this polymer can be easily
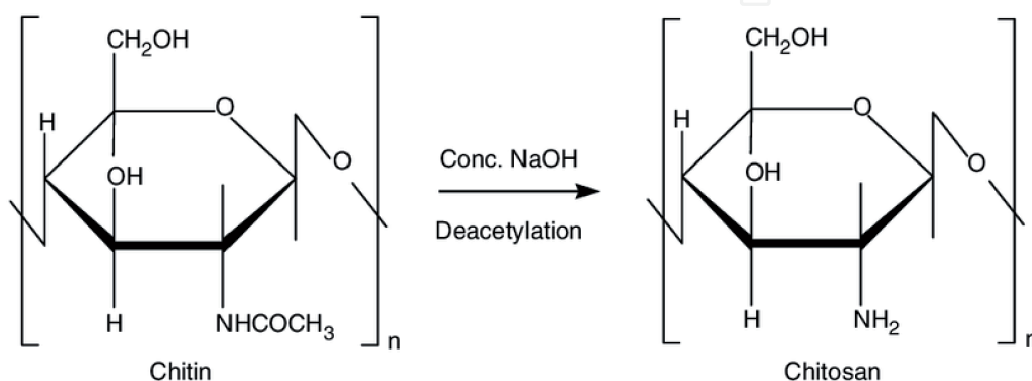

Figure 1.
The N-deacetylation of chitin into chitosan.

dissolved in pH below 6.3. However, both chitosan and chitin are insoluble in an aqueous medium.

Chitin or chitosan is highly available from different species of shrimps, prawns and crabs. These seafood shells release chitosan, which shows properties of antimicrobial and antioxidant activity.

1.2 Characterisation of chitin and chitosan

One of the differences between chitosan and chitin is the presence of amino groups. Amino group in chitosan exhibits high solubility in acidic medium and able to form complexes with metal ions. These positive charges interact with drugs and physiological barriers in the GIT, which is useful in the formulation design of the drug delivery system [9].

Some factors affect chitosan properties, including the degree of deacetylation, degree of substitution, and molecular weight [9, 11]. These factors should be considered before using chitosan as a polymer in a drug delivery system. Most of the chitosan applications are affected by these factors through intermolecular or intramolecular hydrogen bonds [12].

1.2.1 Degree of deacetylation and molecular weight of chitosan

The degree of chitosan deacetylation will affect its biological activity, including swelling rate, molecular weight, crystallinity and polydispersity. The deacetylation process leads to the protonation of the amino groups [13]. A highly positive charge will improve the activity of chitosan as mucoadhesive permeation enhancing [14] and haemostatic agent [15]. Sometimes, the degree of deacetylation can be used to estimate the water solubility of chitosan [11] as shown in **Table 1**.

The degree of deacetylation can influence the particle size and molecular weight of chitosan [13]. The removal of the acetyl group in the structure of chitosan or chitin from deacetylation reduces the interaction between molecules. A low number of acetyl groups minimises the chain length, thus reducing the molecular weight of the polymer [16].

The molecular weight of the polymer will influence the degree of swelling [17]. High molecular weight chitosan (HMWC) tends to have a higher cross-linking ability. Therefore, the drug-coated with HMWC tends to release more slowly [18]. This characteristic is favourable in sustained-release oral drug delivery.

Generally, the lower the molecular weight, the higher solubility of chitosan is obtained [13, 19]. HMWC appears in α-chitin crystalline or antiparallel structure. The structure forms after the release of water, which leads to the loss of entropy during aggregation of the polymeric chain [13]. This phenomenon results in the loss of Gibbs free energy. Gibbs free energy (G) is a way to predict the amount of

Degree of deacetylation	Level	Water solubility
55–70%	Low	Completely insoluble
70–85%	Middle	Partly dissolved
85–95%	High	Good solubility
95–100%	Ultrahigh	Completely soluble

Table 1.
Relationship between degree of deacetylation of chitosan and their water solubility [11].

usable energy in the system. Loss of energy means the reaction in the system tends to be spontaneous.

The α-chitin crystalline form exhibit lower water solubility as compared to β-chitin. The shorter polymeric chain of low molecular weight chitosan (LMWC) is unlikely to aggregate [11]. Interaction between molecules declines due to the formation of the hydrogen bonds is limited. A short chitosan chain contains a low number of amino groups [20].

1.2.2 Crystalline structure

Chitin exists in three different polymorphic forms, which are α-chitin, β-chitin, and γ-chitin (**Figure 2**). The interaction between C=O···NH and C=O···OH maintaining the strength of the polymeric network chain [13]. α-chitin appears in its antiparallel structure and the chain is interacting through both inter-and intramolecular hydrogen bond. β-chitin has a parallel structure, which leads to the formation of the intramolecular hydrogen bond. γ-chitin consists of both antiparallel and parallel structure, as it is the combination of α-chitin and β-chitin [21].

β-Chitin exhibits better water solubility but less common as compared to α-chitin [14]. It has been shown that α-chitosan has a higher crystallinity index as compared to β-chitin. However, the crystallinity index for both forms is lower than the raw chitosan [21]. The crystallinity index will increase when the degree of deacetylation of chitosan increases [16].

The β-chitin has a high affinity to the organic solvent due to its structural flexibility [14]. It exhibits higher reactivity than α-chitosan due to a lack of hydrogen bond. This form has a high capability to swell between crystalline structures while losing its crystalline fraction [21]. The swelling of β-chitin sometimes disrupts the polymeric chain and crystalline structure.
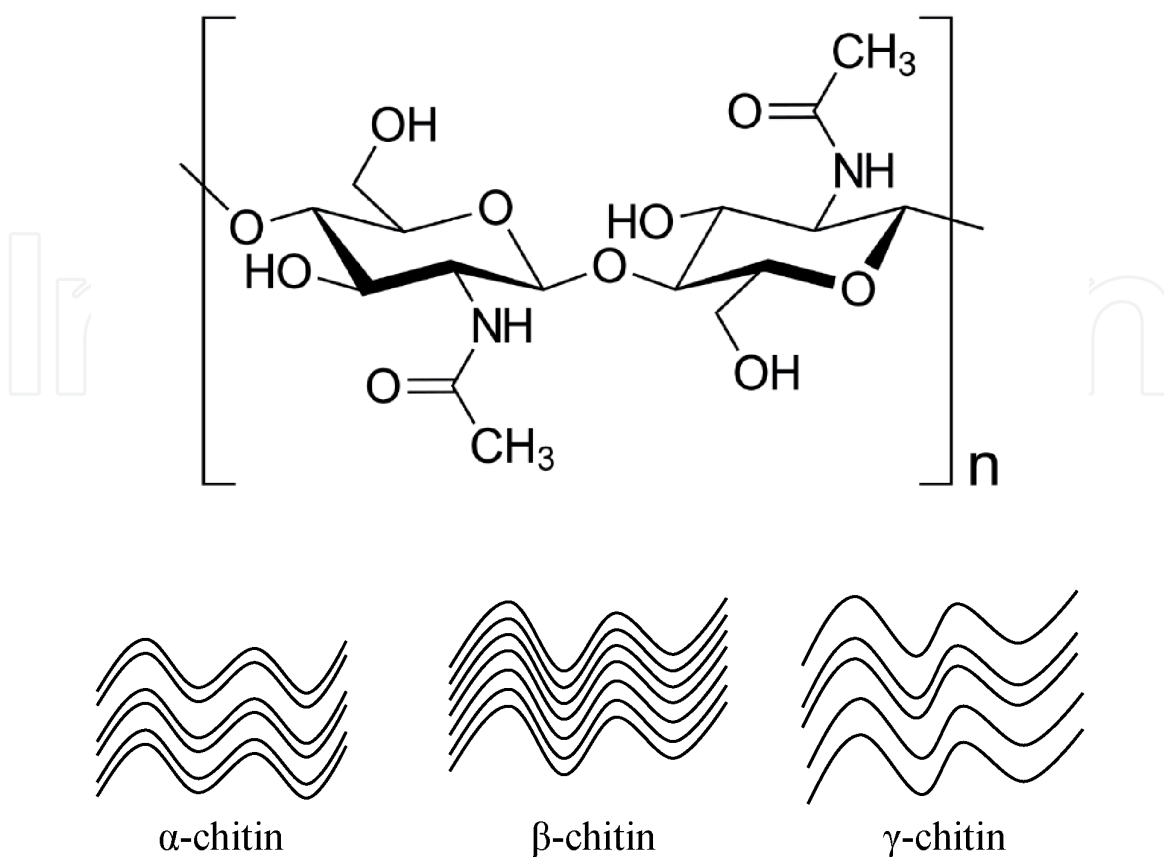

Figure 2.
The different conformational structure between α-chitin, β-chitin and γ-chitin (adapted from [14]).

1.2.3 Polydispersity and particle size of chitosan

The particle size of chitosan plays a major role in developing an efficient carrier for peptide drugs [22]. Monodisperse preparation of nanoparticles is desirable to provide better bioavailability and low toxicity [23]. Polydispersity leads to a larger size distribution, which interferes with the tendency for the nanocarrier to accumulate in the target tissue [23].

Polydispersity describes the degree of non-uniformity of size distribution between molecules due to the aggregation or agglomeration of the polymeric network. It can be estimated using the polydispersity index (PDI), where the ideal index for chitosan nanoparticles is below 0.3 [22, 23]. The degree of deacetylation and molecular weight of chitosan have been proven to influence the polydispersity of the system [24].

PDI increases with an increase in molecular weight. However, it decreases as the degree of deacetylation increases [23]. The increase of amino group protonation and removal of the acetyl group from chitosan structure lead to the enhancement of repulsive forces between molecules and stretch the chitosan to become larger in size [11, 24]. Therefore, the development of chitosan with the optimum degree of deacetylation is needed to minimise the risk of polydispersity. This can be achieved by modifying the time and temperature of the de-*N*-acetylation process [18].

The degree of entanglement for HMWC nanoparticles is higher than LMWC. Therefore, HMWC nanoparticle has a high tendency to aggregate with each other and disrupt the uniformity of the system [25]. However, LMWC cannot be loaded into nanoparticles with smaller size due to its limitation to entangle to the structures of the system [26]. Therefore, maintaining the particle size of the chitosan is crucial in the development of a chitosan nanoparticle.

1.3 Modification of chitosan as biomaterial

Modified chitosan shows greater advantages as compared to unmodified chitosan. The modification of chitosan either chemically or physically may improve its solubility, properties of gelling, and biocompatibility. This modification can be done through cross-linking or substitution [27]. The presence of the various reactive functional groups in the chitosan structure makes it available in many derivatives with different stability properties.

1.3.1 Quaternisation

A quaternary ammonium salt is a hydrophilic group with a permanent positive charge. Therefore, quaternary chitosan does not need an acidic condition to undergo protonation [12, 28]. It allows chitosan quaternary ammonium salts to be soluble in both acidic and basic pH. This is a good approach to increase chitosan solubility in water [28].

The high strength of the positive charge will weaken the hydrogen bond. However, this activity depends on the degree of substitution. The higher the degree of substitution, the higher the water solubility of chitosan. This will improve the quality of chitosan to act as a mucoadhesive agent that aids the penetration into mucus [29].

Trimethyl chitosan (TMC) is an example of a chitosan derivative from quaternisation. This modification is effective in enhancing the bioavailability of antibacterial drugs with antibacterial properties. Moreover, quaternised chitosan also exhibits antibacterial properties by the interaction of its positive charge with the negative charge of Gram-negative bacteria [28–30].

1.3.2 Sulfonated chitosan

Sulfonated chitosan is water-soluble anionic chitosan, which was derived with N-benzyl disulfonated derivative [31]. This modification of chitosan has been shown to be effective, not only as antiviral and antibacterial but also as anticoagulant properties. Sulfonated chitosan interferes with the interaction between the envelope glycoprotein (gp120) and its receptor on the CD4 cells' surface. Therefore, It inhibits the replication of HIV [32].

Sulfonated chitosan has been developed to carry anticoagulant drugs such as reviparin and enhance the anticoagulant activity. Sulfonated chitosan nanoparticles interact with factor Xa and inhibit their function in the blood clotting mechanism [31, 33]. Low molecular weight sulfoethyl chitosan acts as capping of nanoparticles [33]. A capping agent is needed in the nanoparticulate system to prevent agglomeration.

Amphotericin B is used to treat fungal infection by binding to ergosterol on the cell membrane of fungal. It depolarises the membrane and alters its permeability [34]. Sulfonated chitosan has been used in the formulation of amphotericin B to reduce the side effects of the drug by making sure the drug specifically targets the ergosterol of fungal [35].

1.3.3 Thiolation

The structure of mucin that coats the intestinal epithelial cell contains the cysteine-rich domain. This domain easily forms a disulfide bond with a thiolated derivative of chitosan. The bond formations increase the residence time for the chitosan to the mucus and increase the mucoadhesive property of the chitosan [30, 36]. When chitosan covalently bonded with any thiolated moiety, water-soluble carbodiimide is required as a cross-linker [30]. Carbodiimide increases the number of thiol groups. This enhances the immobilisation phenomena, which is the formation of disulfide bonds due to the activation of carboxylic groups.

The thiolation of TMC with the conjugation of cysteine residue increases the strength of covalent bonding between mucin. The covalent bond formation of chitosan with thioglycolic acid (TGA) is an effective carrier in delivering trimethoprim for urinary tract infection [37]. The preparation of chitosan-TGA nanoparticles should be stabilised by covalent cross-linking with polyanion, such as tripolyphosphate [38]. The cross-linking minimise the risk of particle aggregation, increases the disulfide bond, and strengthens the mechanical force between networks, which allows trimethoprim to be released slowly [37].

The conjugation of chitosan with glutathione will protect peptide drugs from aminopeptidase in the GIT [39]. Glutathione has thiol groups which exhibit strong electron-donating properties. It forms an α-peptide bond with cysteine moiety of aminopeptidase [30]. Glutathione also acts as an antioxidant which reduces oxidative stress and increases the adhesion of formulation to the cell [30].

1.3.4 Carboxy alkyl chitosan

The poor water solubility of chitosan makes them less effective as a permeation enhancer. The addition of the carboxyalkyl group will transform the molecule into amphoteric in nature and allow them to react in both basic and acidic conditions [40]. The interaction between the carboxyl group and the primary amino group of chitosan exhibits a promising approach in developing controlled drug release.

The Schiff base reactive gives rise to the formation of the N-carboxymethyl derivative of chitosan [41]. This modification of chitosan has been shown to improve

the absorption of low molecular weight heparin (LMWH). After coating into polydopamine, the conjugation of LMWH with carboxymethyl chitosan into polyurethane substrate shows excellent hemocompatibility of heparin. This modification enhances the bioavailability and improves the anticoagulant effect of heparin [42].

Propranolol hydrochloride has a short half-life and requires every 6 to 8 hours in a divided daily dose. The use of carboxymethyl chitosan will coat the drug with a polymer matrix. It controls the release of drug with zero-order kinetics, allowing the constant amount of drug will be eliminated per unit time. The hydration of carboxymethyl chitosan will form a gel layered around the drug, which essential in drug release [43, 44].

1.4 Release mechanisms of chitosan nanoparticle

The release of drugs from the chitosan nanoparticle is influenced by the hydrophilicity of chitosan and pH of the swelling solution. Chitosan release mechanism involves swelling, diffusion of drugs through the polymeric matrix and polymer erosion [45]. Due to the hydrophilicity of chitosan, chitosan nanoparticles exhibit pH-dependent drug and controlled drug release system [6].

Acid and base act as catalysts in the degradation of polymers [46]. Therefore, the behaviour of swelling and the amount of drug released is highly dependent on the pH of the swelling solution. Hence, a modified drug release can be achieved [46]. When polymers get into contact with an aqueous medium, the water will diffuse into the polymer until the polymer swells (**Figure 3**).
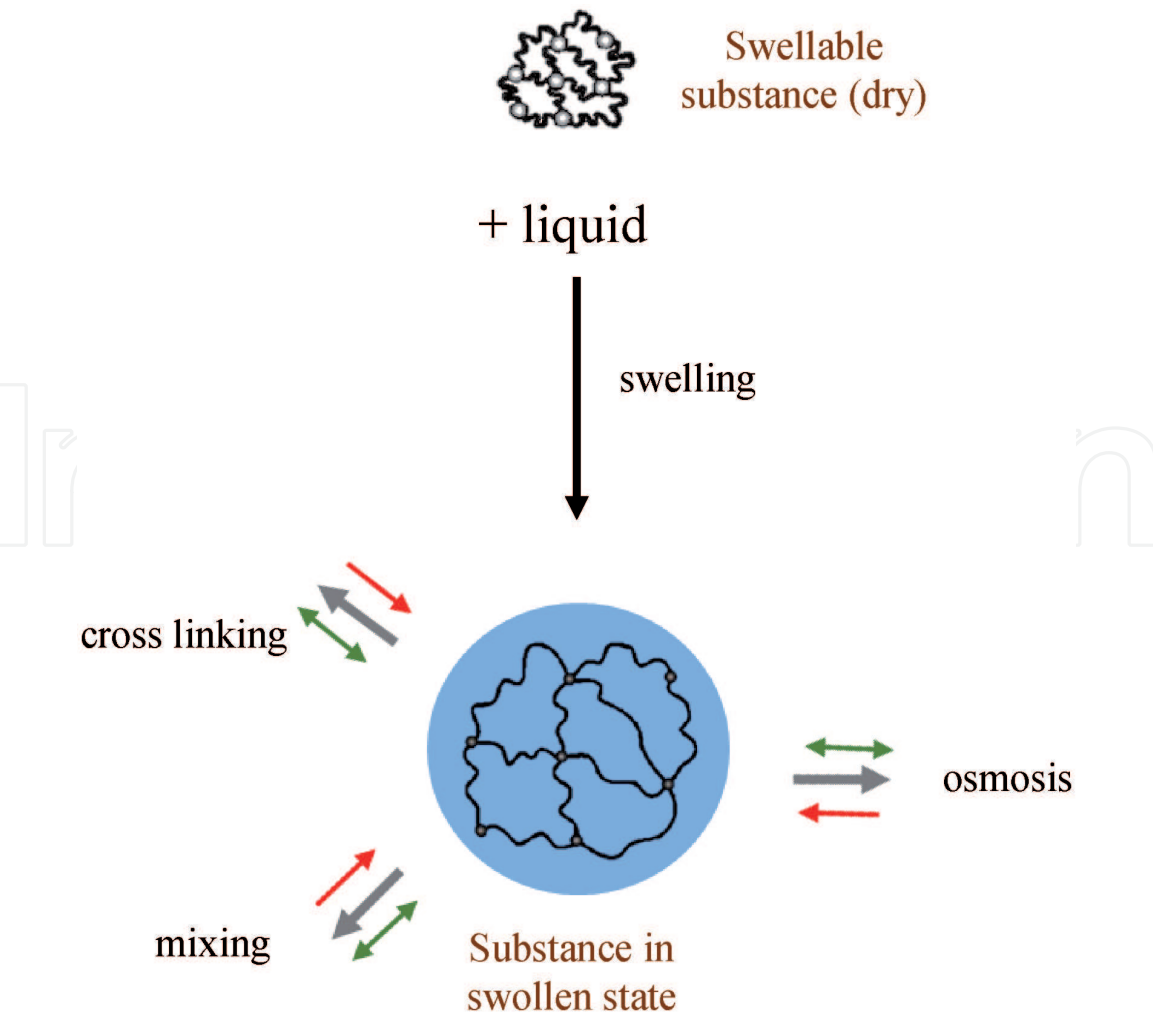

Figure 3.
The swelling of swellablepolymers in aqueous medium (adapted from [49]).

The polymeric chitosan chain will start to detangle. The swelling polymer will form pores which allow drugs to diffuse out of the nanoparticulate system [6, 43, 47]. Therefore, the water solubility of chitosan is crucial in the mechanism of drug release from the nanoparticulate system.

1.5 The use of chitosan to improve drug delivery system

Oral drug administration is the most convenient route, especially among the elderly and children. Unfortunately, some drugs and vaccines cannot withstand the physiological barrier of GIT. In the presence of mucus, proteolytic enzymes, and first-pass metabolism by the liver, drugs tend to be degraded or converted into inactive metabolites [48]. Some drugs will be excreted in the urine lead to low bioavailability.

Due to the challenges aforesaid, chitosan and its derivatives have been used in the development of nanotechnology to improve oral drug delivery [25, 30]. It encapsulates drugs to protect them from degradation in the GIT environment. As a consequence of its excellent biodegradable, biocompatibility, and non-toxic properties, chitosan promotes a stimuli-responsive release of drugs. It allows active ingredients to be released from the formulation in a controlled manner, specifically in enteric-coated drugs [38, 43].

Due to its antimicrobial properties, it was used in the delivery of oral antibiotics to eradicate Gram-negative bacteria such as *E. coli* [49]. This approach not only improves the bioavailability of antibiotic in the body but also indirectly enhance the effectiveness of the drugs in eradicating the infection [15].

2. The properties of protein and peptide

A peptide is made up of short polymers of α-amino acid, which is around 20 to 50 amino acids. The function of small peptides depends on the functional group of various amino acids. Examples of active peptides are glutathione, bradykinin, angiotensin, vasopressin and oxytocin [4].

Protein is a macromolecular and high molecular weight polypeptide, which is made up of long-chain amino acids (more than 50) arranged in a linear chain through peptide bonds [50]. It can exist in four different structural conformations such as primary, secondary, tertiary, and quaternary. The formation of these structures is dependent on the intermolecular interaction between functional groups of amino acids [51], through covalent bonds or non-covalent bonds.

The covalent bonds are strong bonds which include peptide bonds and disulfide bonds [51]. Peptide bonds are interactions between two consecutive amino acids through amino and carboxyl groups. Meanwhile, disulfide bonds link two cysteine residues through sulphhydryl groups [52].

On the other hand, non-covalent bonds are weak bonds that include hydrogen, electrostatic and hydrophobic bonds. Hydrogen bonds link two different peptides with the hydrogen atom of the N-H group and oxygen of the carboxylic group. Hydrophobic bonds will occur if the hydrophobic nature between non-polar side chains of amino acid interacts with each other [51].

3. Oral peptide delivery

The physiological barriers in the GIT responsible for protecting the body from the entry of pathogens. These barriers may reduce the bioavailability of the

protein. The barriers aforesaid include biochemical, cellular, and mucus barriers (**Figure 4**) [53].

The entire GIT has been coated with mucus. Mucus also promotes a physical barrier between the lining of epithelial and lumen [54]. It contains mucin protein which secretes proteolytic enzymes and traps peptide drugs through electrostatic interaction [55].

The epithelium of the GIT consists of an intestinal epithelial stem and microfold cells (M-cell) [48]. These cells are responsible for controlling protein uptake from the gut lumen into the bloodstream. Since protein drug is a macromolecule, the presence of protein complexes between adjacent epithelial cells prevents paracellular transport of drug [56]. Meanwhile, transcellular transport is limited only to highly lipophilic molecules, unless the transportation is mediated by P-gp [57].
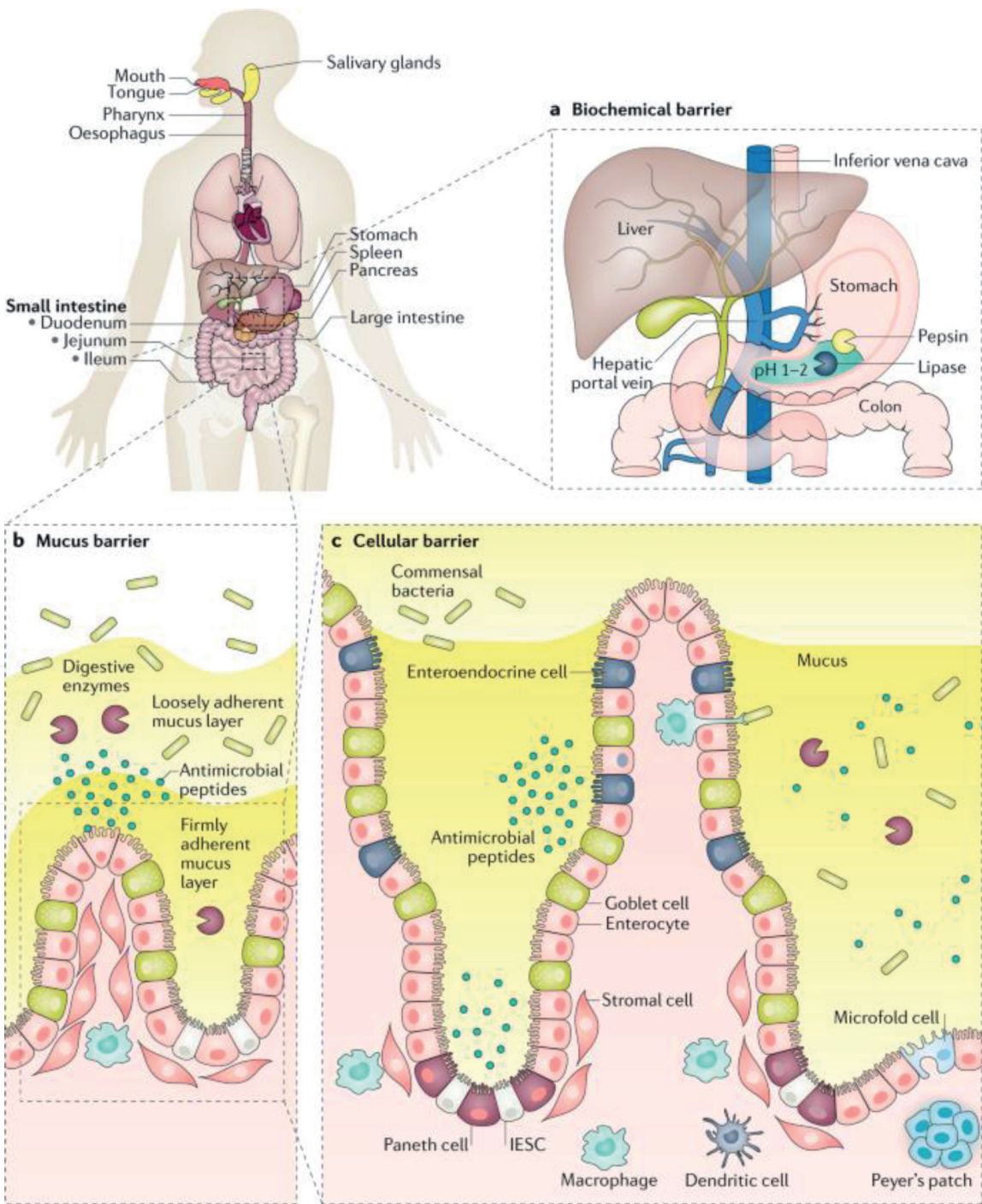

Figure 4.
Physiological barriers to oral protein and peptide delivery (adapted from [53]).

Due to the physical and chemical instability of protein in the GIT, they would not achieve an acceptable therapeutic bioavailability. The nature of the GIT as great physiology to digest food will be a barrier for protein drugs to penetrate through the membrane. The challenges and strategies to improve the protein drug delivery through oral administration need to be considered to ensure the drug achieves adequate therapeutic concentration in the body [58].

3.1 Challenges in oral peptide delivery

3.1.1 The presence of proteolytic enzyme and pH of GIT

GIT is the hollow organs include the oral cavity, stomach, small intestine, large intestine, and colon. Each part of the GIT is varying in pH. However, most proteins are stable at neutral pH and tend to undergo protein denaturation at the extreme changes in pH [48]. A building block of protein is sensitive to pH. In the presence of hydrochloric acid in the stomach, hydrolysis will occur and the disulphide bonds of peptide will be reduced.

Acidic pH environment activates the conversion of pepsinogen into pepsin [59], by transferring hydrogen ion (H+). Pepsin is responsible for the breakdown of peptide bonds which will interfere with the structure and stability of the peptide [59].

In the small intestine, the pancreatic juice is secreted in the duodenum. This juice contains pancreatic enzymes and bicarbonate ions (HCO_3^-) [48]. The pancreatic enzymes consist of amylase, lipase, and protease, which are responsible for the digestion of lipid and peptide. Protease catalyses the proteolysis rate, which cleaves peptide bonds through hydrolysis [4, 58].

The presence of chymotrypsin and peptidase in the jejunum interferes with peptide absorption in the epithelial membrane [48]. Peptide drugs are digested before it reaches the membrane and the fraction of the undigested peptide will reduce. This physiological function will lower the possibility for the therapeutic concentration of peptides to be achieved in the systemic circulation [58].

3.1.2 The intestinal barrier to drug absorption

The layers of the epithelial cell of the intestine are covered by mucus or mucin glycoproteins [55]. These glycoproteins will form a gel layer that covers the surface of the intestinal cell. The diffusion rate for the peptide to the epithelial membrane is restricted in the presence of mucus [54].

One of the intestinal mucosal epithelial cells is the goblet cell. Goblet cells are responsible for secreting mucin 2 in the intestine and Mucin-5b in the colon. Lubricate the passage for chime is the main function of the mucus layer, protecting the epithelium from mechanical damage of GIT [54].

The overexpression of mucin will interfere with the pharmacokinetics of drugs. The higher the concentration of the mucin, the lower the ability of a drug to diffuse through the membrane. Drugs and mucin interact through hydrophobic and Van der Waals interaction.

Large amounts of enzymes present within the mucus layer increase the tendency to digest peptide drugs [58]. The ionic strength, pH, and chyme content in the intestine will affect the charge density in the mucin [54]. The presence of a charged group on mucin interact ionically with charged particles and immobilised them in the mucus. The immobilisation of peptides leads to the clearing from the tract when the layer of mucus is shed [60].

3.1.3 Tight junctions between adjacent epithelial cells

Tight junctions are protein complexes that existed within the adjacent epithelial cells [48]. It prevents leakage and restricts the flux of substances through the paracellular pathway [61]. They consist of transmembrane protein with extracellular domains called Claudin 4 (CLDN4). CLDN4 protects the paracellular physiological function of GIT [62].

The linkage of CLDN4 domains with zonula occludens 1 will result in the connection of cytoskeleton components through linker protein [62]. The components include actin, myosin and microtubules which involve in the contraction of muscle, upon phosphorylation. The contraction leads to cellular tightness, hence, reduce the permeability of substance into the cell.

3.2 Strategies to improve oral peptide delivery system using chitosan

The effective delivery of oral peptide drugs can be achieved by altering the formulation for maximum solubility, avoid enzymatic degradation and enhance the absorption of drugs through the intestinal epithelial cell [63]. For the sake of preventing enzymatic degradation or inactivation, the addition of enzyme or protease inhibitor is a great approach (**Figure 5**). Proteolytic enzymes are responsible for cleaving protein molecules into an inactive amino acid chain. Protease inhibitors such as aprotinin and chromostatin can be used to prevent the inactivation of protein drugs [7].

As discussed earlier, protein molecules show poor permeability through various mucosal surfaces and biological membranes. The improvement of membrane permeability can be achieved by the inclusion of a permeation enhancer into the formulation. Permeation enhancers are either tight junction selective or membrane perturbing [61].

Chitosan and its derivatives have been used as an enzyme inhibitor, permeation enhancer and mucoadhesive agent [30]. With the different mechanism, modification, and preparation technique, this polymer also involves in the encapsulation of peptide drugs into the nanoparticulate system [60], which protect them from harsh GIT environment.
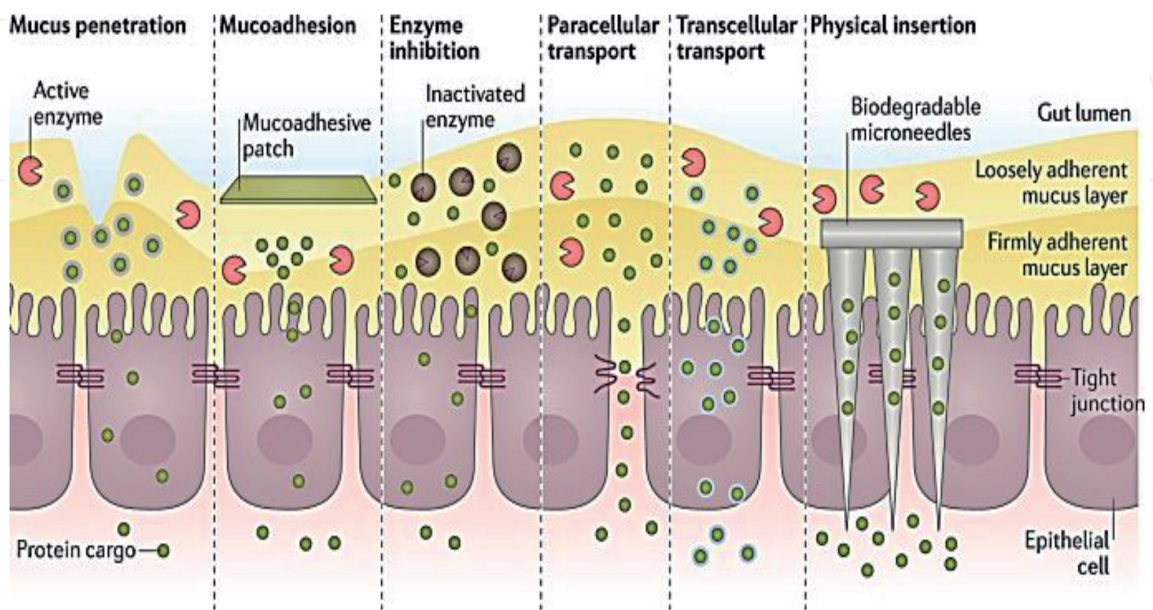

Figure 5.
Strategies to improve oral peptide delivery system (adapted from [53]).

3.2.1 Enzyme inhibition

There are two types of protease, including serine protease and Zn^{2+}-dependent protease. Serine proteases, such as trypsin, chymotrypsin and elastase are pancreatic digestive enzymes. Meanwhile, Zn^{2+}-dependent protease such as matrix metalloproteinase is an insulin-degrading enzyme [64]. Some enzyme inhibitors need chitosan to enhance their anti-protease activity and minimise peptide drug degradation [64]. For example, chymostatin is a protease inhibitor selectively to chymotrypsin-like serine proteases. This inhibitor will covalently be linked to the amino group of chitosan.

The active site of matrix metalloproteinase, such as carboxypeptidase, contains Zn^{2+} binding motif. It requires Zn^{2+} to promote nucleophilic attack by water. This protease cleaves membrane-bound pre-proteins of the cell to release cytokine. The ethylenediaminetetraacetic acid (EDTA) is a complexing agent that is capable of forming a complex with Zn^{2+} and retard the nucleophilic attack of water on carboxypeptidase. To inhibit the Zn^{2+}-dependent protease, the EDTA is covalently bound to the primary amino groups of the chitosan-inhibitor conjugate [27, 65].

Moreover, the study showed that the effect of trypsin inhibitors would be disrupted after the gastric phase. Therefore, the encapsulation of the peptide drug and trypsin inhibitor with chitosan-EDTA conjugates improve the controlled release of the molecules.

3.2.2 Chitosan as a mucoadhesive agent

Chitosan derivatives improve the permeation of water-soluble drug molecules due to their ability to adhere to the mucus [30]. Thiolated chitosan shows a greater effect in improving drug permeation through the cell membrane. With the formation of disulfide bridges, the thiol group of chitosan interacts with the cysteine-rich subdomains of mucus and allows greater mucoadhesion. Thus, the absorption of the peptide drug molecule increases with residence time [30].

It is worth noting that chitosan with a low degree of acetylation and high molecular weight leads to high charge density. The higher positive charge density of chitosan will bind to negatively charged tight junction channels. Ion displacement occurs, leading to intracellular spaces loosening [16].

The integrity and permeability of tight junctions can be illustrated with transepithelial electrical resistance (TER). The ability of TMC [66] and carboxymethyl chitosan [67] in decreasing the TER will increase the permeability of peptide drugs. TMC has been used to formulate buserelin, a synthetic peptide analogue for LHRH agonist, by the oral delivery system [68].

Thiolated chitosan, such as glutathione, cysteine and N-acetylcysteine, have strong mucoadhesive properties due to covalent bonding with cysteine-rich subdomains of the mucus glycoprotein. For chitosan-glutathione, this derivative improves chitosan stability, enhanced mucoadhesion and permeation enhancing effect. This system has been applied to the oral delivery of immunostimulant drug, thymopentin [30].

Chitosan-cysteine shows similar mucoadhesive but improved cohesion as compared to unmodified chitosan. The cohesiveness of polymeric drug formulation is crucial to ensure the stability of the drug and will be released in a controlled manner. Furthermore, chitosan-N-acetylcysteine produces a longer retention time than unmodified chitosan. However, no drugs have been tested yet for these two chitosan derivatives [30].

3.2.3 Encapsulation of peptide into the nanocarrier

By encapsulating peptides into a nanoparticulate system, enzymolysis and peptide aggregation can be avoided. This approach enhances the absorption of peptide drugs through the transmembrane of the small intestinal epithelium [69]. Nanoparticle will provide controlled-release properties in the presence of chitosan as a polymer. This condition will reduce repetitive dose administration and improve drug bioavailability [43]. In the presence of chitosan as a mucoadhesive agent, the retention time between formulation and absorption site will be maximised.

Cyclosporine is a cyclic peptide drug used to suppress the immune system, after organ transplantation. Cyclosporine with high molecular weight (1.32 kDa) shows poor bioavailability with low permeability through the biological barrier. Conventional oral cyclosporine has been shown to have an unpredictable low therapeutic concentration in the bloodstream.

Therefore, a nanoparticle drug delivery system is a promising strategy to improve the oral bioavailability of cyclosporine. Chitosan nanoparticles in the presence of tripolyphosphate, as a cross-linker, make it more convenient as compared to conventional ones. The bioavailability of the nanoparticulate cyclosporine increases by 73% [69]. Exendin-4 is a glucagon-like peptide-1 receptor agonist that has been approved to control type 2 diabetes mellitus. This peptide drug has high susceptible to enzymatic degradation [70]. Chitosan-tripolyphosphate conjugated nanoparticle was used to design oral suspension and enteric-coated capsules of exendin-4 to increase the bioavailability of exendin-4 slightly.

3.2.4 Efflux pump inhibition

Efflux pump is a membrane protein located within the cytoplasmic membrane of a cell. It translocates a variety of substrates across extra- and intra-cellular membranes. Multidrug efflux pump can be one of the drug resistance mechanisms, as it pumps foreign substances (or drugs) out of cells. This active process is an ATP-dependent [71].

P-glycoprotein (P-gp) is a transmembrane glycoprotein and the best example of a multidrug efflux pump. It is expressed and located in the intestinal epithelium, liver cells and proximal tubule cells of the kidney. P-gp is also located within the blood–brain barrier (BBB), which provides an obstacle for drugs to enter the region. Therefore, it must be difficult for antipsychotic drugs to bypass BBB and exert their effect [57, 71, 72].

Chitosan may enhance drug permeation by opening of tight junctions which is highly related to CLDN4 [73]. Chitosan will modulate CLDN4 protein redistribution to the cytosol and disrupt tight junctions [62]. This phenomenon will enhance paracellular permeability and reduce TER. Thus, declining barrier function of epithelial cells to allow drugs to enter the cell [74].

Furthermore, the use of thiolated chitosan (thiomer) has shown to be useful in bypassing the P-gp. The thiol-moiety of thiolated chitosan may allow the formation of disulfide bonds between the cysteine groups of the P-gp [9]. The thiomers then enter the channels of the P-gp pump together with the therapeutic agent, which obstruct the function of the multidrug efflux pump [57].

For infectious disease by Gram-negative bacteria, chitosan plays an important role in facilitating effective delivery of antimicrobials to the infection site [15, 49]. Chitosan will encapsulate drugs and carry them into bacterial cells by attraction forces between polycationic chitosan and negatively charged bacteria [49]. This action avoids the efflux pump at the cell membrane of the bacterial cell.

4. Oral vaccine delivery system

Vaccination is one of the most cost-effective approaches to prevent infectious diseases such as hepatitis B, tetanus, polio, and rabies. Vaccines contain pathogens, either live-attenuated, inactive or killed antigen [75]. These pathogens will be administered in the body and recognised by the immune system.

The oral delivery of vaccines is quite challenging as the pathogen is introduced into the body. It is mandatory to ensure mucosal immune response works effectively to protect the body against the pathogen and their toxin [54].

As the vaccine enters the intestine, its presence will trigger the inductive site, the Peyer's patches. The Peyer's patches consist of M-cell which will allow the entry of the antigen through endocytosis. The antigen then will be transported into intraepithelial dendritic cells or macrophages and be taken up by the cell through phagocytosis [76].

The antigen-loaded dendritic cell will present the antigen fragment on its surface and triggers the activation of naive CD4+ T-cells. The activated CD4+ T-cells will bind to the antigen fragment, MHC class II. This binding releases chemical mediators, interleukin-2 (IL-2), that function to regulate the activity of lymphocytes for immunity. IL-2 stimulates the cell division of CD4+ T-cells, activates B-cells and cytotoxic T-cells. B-cell is responsible for mediating humoral immunity by differentiating into plasma cells. Plasma cells will generate antibodies to fight against pathogens [77].

5. Challenges in oral vaccine delivery system

Viral protein requires the right structural conformation to attach to the host cell and replicate. Highly acidic in the stomach and extreme temperature changes will cause protein denaturation. The denaturation of the virus will alter the conformation of its structure [58]. The high temperature will break the phosphodiester bond. However, at low temperature, the degradation of the nucleic acid will also lead to viral inactivation [78].

Furthermore, to transport vaccines orally, it should be able to overcome the biological barrier of the intestinal epithelial cell such as tight junction and mucus. The hydrophilic antigen cannot cross the phospholipid bilayer to enter systemic circulation due to the function of tight junction in controlling the permeability of the membranes. Therefore, the uptake of the antigen to mucosal tissue is limited with a short time of exposure [78].

The GIT contains normal flora or microbiota which help in maintaining the structure of the gut mucosal barrier [55]. Those microbiotas not only aid nutrient metabolism, but they also possess an action to protect against invading pathogens [79, 80]. Well-balance microbiota is needed to induce the effectiveness of vaccines through oral administration. The delivery of the vaccine will be interrupted in patients with microbiota dysbiosis, leading to blunted vaccine response [80].

The induction of danger signals appropriately by the vaccine is essential to trigger an immune response [81]. Due to these limitations, there is a problem in inducing an adequate immune response against administered pathogens [3]. Consequently, a higher and repetitive dose is required. Nevertheless, the administration of high antigen doses repetitively may develop systemic oral tolerance towards vaccines [3, 78].

6. Strategies to improve oral vaccine delivery system using chitosan

The uptake of antigen by immune cells depends on the particle size of the antigen. The smaller size of pathogens is readily taken up by dendritic cells [75]. The same goes for peptide, encapsulation of pathogens in nanoparticles is a good approach to improve the effectiveness of vaccines in stimulating the immune system [75].

Small particle size is required to penetrate the mucus. The formulation will be excluded out from the layer of mucus if particle size greater than normal mucus pore size (100–500 nm). This leads to the interruption in the bioavailability of antigen to targeted antigen-presenting cell (APC). Therefore, the development of nanoparticulate systems is required to provide a smaller size (20 – 40 nm) of formulation [82].

Medium molecular weight chitosan (MMWC) with the degree of deacetylation of 85% has been shown to improve the delivery of ovalbumin antigen with the presence of alginate and calcium phosphate (CaP). CaP has adjuvant properties by activating the surface expression of B-cell. CaP can be coated with mucosal penetrating polymers, such as chitosan and alginate to avoid biodegradation by enzymes present in the GIT [82]. In the stomach, the alginate-chitosan-coated CaP nanoparticle delays the release of ovalbumin antigen. The antigen then will be released in the intestine and colon with a sustained-release mechanism. This nanocarrier has successfully encapsulated ovalbumin antigen with small size (< 50 nm) [82].

The antigen should be transported to the intestine and directly to the M-cell of the Peyer's patches [6]. Chitosan develops well-protected mucoadhesion by prolonging the residence time at mucosal surfaces. The uptake of antigen by epithelial cells of the intestine will be improved by chitosan. An increase in the activity of macrophages will improve the secretion of mucosal IgA and IgG [76].

7. Conclusions

Chitosan-based drug formulation has gained attention for their ability to serve as a carrier and an enhancer for oral delivery of peptides and vaccines. Although oral delivery is the most convenient and preferred route of administration, however, it has limitations due to the presence of the proteolytic enzyme, pH of GIT and the intestinal barrier to drug absorption. In recent years, there has been considerable research interest in the application of chitosan as an enzyme inhibitor, mucoadhesive agent and efflux pump inhibitor. Interaction of positively-charged amino groups of chitosan with negatively-charged sialic acid groups that exist in mucin prolongs the residence time between drugs and membranes, therefore enhancing the bioavailability of the drugs. Other formulation strategies include encapsulation of proteins, peptides and vaccines into a nanoparticulate delivery system. By encapsulating peptide into a nanocarrier system, the enzymolysis and peptides aggregation can be avoided thus enhances the absorption of peptide drugs in the intestinal epithelium. Similarly, encapsulation of pathogens in nanoparticles is a good approach to improve the effectiveness of vaccines in stimulating the immune system.

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Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

kDa	Kilodalton
nm	Nano-metre
GIT	Gastrointestinal tract
G	Gibbs free energy
PDI	Polydispersity index
LMWC	Low molecular weight chitosan
HMWC	High molecular weight chitosan
MMWC	Medium molecular weight chitosan
TMC	Trimethyl chitosan
TER	Transcellular electrical resistance
TGA	Thioglycolic acid
LMWH	Low molecular weight heparin
P-gp	P-glycoprotein
CLDN4	Claudin 4
EDTA	Ethylenediaminetetraacetic acid
IL-2	Interleukin-2
APC	Antigen-presenting cell
CaP	Calcium phosphate
PEG	Polyethylene glycol
PGC	Pegylated chitosan
HMPCP	Hydroxypropyl methylcellulose phthalate

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