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Chitosan and Its Derivatives for Drug Delivery Perspective

T.A. Sonia and Chandra P. Sharma

Abstract Biopolymers are promising materials in the delivery of protein drugs due to their compatibility, degradation behavior, and nontoxic nature on administration. On suitable chemical modification, these polymers can provide better materials for drug delivery systems. Nanostructured drug carriers allow the delivery of not only small-molecule drugs but also of nucleic acids and proteins. The use of biopolymers like dextran, starch, alginate, and pullulan nanoparticles in drug delivery are briefly discussed. Being the only cationic polysaccharide of natural origin, chitosan, a versatile biopolymer of the aminoglucopyran family is being extensively explored for various biomedical and pharmaceutical applications such as drug delivery. In this review, we aim to comprehensively integrate the recent applications of chitosan nano/microparticles in oral and/or buccal delivery, stomach-specific drug delivery, intestinal delivery, colon-specific drug delivery, and gene delivery, giving special emphasis to oral drug delivery.

Keywords Chitosan · Drug delivery · Mucoadhesion · Nanoparticles · Thiolated chitosan

Contents

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1 Introduction

Nanotechnology is being increasingly explored in science and industry for widely different applications. Nanotechnology and polymers have captivated a tremendous interest in many areas such as the pharmaceutical industry and therapeutic innovation among others. Natural and synthetic polymers have been used as a promising tool for nanoscale drug carrier systems, especially in oral administration of poorly absorbed therapeutic drugs [1]. In recent years, great developments have been made in the field of mucoadhesive polymer systems in formulations that increase the residence time of drugs on mucosal membranes and subsequently, enhance the bioavailability of drugs with poor oral absorption [2, 3]. In this review, we will be emphasizing the importance of the mucoadhesive polymer chitosan and its derivatives as a drug carrier, giving special attention to oral protein delivery.

2 Nanomaterials

The rapid expansion of nanotechnology promises to have great benefits for society. Nanomaterial is matter at dimensions of roughly 1–100 nm, where a unique phenomenon offers novel applications. The major advantages of nanoparticles as a delivery system are in controlling particle size, surface properties, and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Although nanoparticles offer many advantages as drug carrier systems, there are still many limitations to be solved such as poor oral bioavailability, instability in circulation, inadequate tissue distribution, and toxicity [4]. For example, their small size and large surface area can lead to particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. In addition, small particle size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available. Nanotechnology is making significant advances in biomedical applications, including newer drug delivery techniques. There has been considerable research in the developing of biodegradable nanoparticles as effective drug delivery systems [5]. The drug is dissolved, entrapped, adsorbed, attached, or encapsulated into the nanoparticle matrix. The nanoparticle matrix can be of biodegradable materials such as polymers or proteins. Depending on the method of preparation, nanoparticles can be obtained with different properties and release characteristics for the encapsulated therapeutic agents [6].

2.1 Nanomaterials in Drug Delivery

Nanoparticles applied as drug delivery systems are submicron-sized particles (3–200 nm), devices, or systems that can be made using a variety of materials including polymers (polymeric nanoparticles, micelles, or dendrimers), lipids (liposomes), viruses (viral nanoparticles), and even organometallic compounds (nanotubes) [7]. Nanoparticles are engineered structures with at least one dimension of 100 nm or less. These novel materials are increasingly used for commercial products, including developing new designs for medicinal application. We are facing tremendous opportunities and challenges in combining emerging nanotechnology with cellular and molecular techniques to develop better diagnosis and therapeutics for diseases such as cancer. However, before these applications are used in clinical practice and commercialized, we have to ensure that they are safe. A number of studies have demonstrated that nanoparticle toxicity is complex and multifactorial, potentially being regulated by a variety of physicochemical properties such as size, chemical composition, and shape, as well as surface properties

such as charge, area, and reactivity [8]. However, there is no regulation or guideline, particularly in toxicology, to guide the research of medicinal application of nanomaterials.

3 Biopolymers in Drug Delivery

During the past few decades there has been an increasing interest in the development of biodegradable nanoparticles for effective drug, peptide, protein, and DNA delivery [9]. Incorporation of the drug into a particulate carrier can protect the active substance against degradation in vivo and in vitro, improve therapeutic effect, prolong biological activity, control drug release rate, and decrease administration frequency [10]. At present, biodegradable polymers like starch, dextran, pullulan, chitosan, and alginate (Fig. 1) are being used to encapsulate proteins and peptides. In this section we will discuss the use of these biodegradable polymers as drug carriers. Although a number of synthetic biodegradable polymers have been developed for biomedical applications, the use of natural biodegradable polymers remains attractive because of their abundance in nature, good biocompatibility, and ability to be readily modified by simple chemistry [11]. A majority of drug delivery systems using natural polymers have been based on proteins (e.g., collagen, gelatin, and albumin) and polysaccharides (e.g., starch, dextran, hyaluronic acid, and chitosan). Polysaccharide-based microparticles have gained much attention in developing controlled-release microparticulate systems because of their flexibility in obtaining a desirable drug release profile, cost-effectiveness, ease of modification by simple chemical reactions for specific applications, broad range of physicochemical properties, and broad regulatory acceptance [12]. Further, they display biocompatibility and biodegradability, which are the basic characteristics for polymers used as biomaterials. Therefore, they are widely used in pharmaceutical formulations and, in several cases, they play a fundamental role in determining the release rate from the dosage form. Polysaccharide biodegradable matrices are of interest because the degradation of natural products like starch occurs naturally in the human body [13, 14]. Applications of proteins to delivery of protein drugs have been limited due to their poor mechanical properties, low elasticity, possible occurrence of an antigenic response, and high cost [15].

3.1 Alginate

Alginate is a nonbranched, high molecular weight binary copolymer of (1-4) glycosidically linked β-D-mannuronic acid and α-L-guluronic acid monomers (Fig. 1b) [16, 17]. The high acid content allows alginic acid to undergo spontaneous and mild gelling in the presence of divalent cations, such as calcium ions.

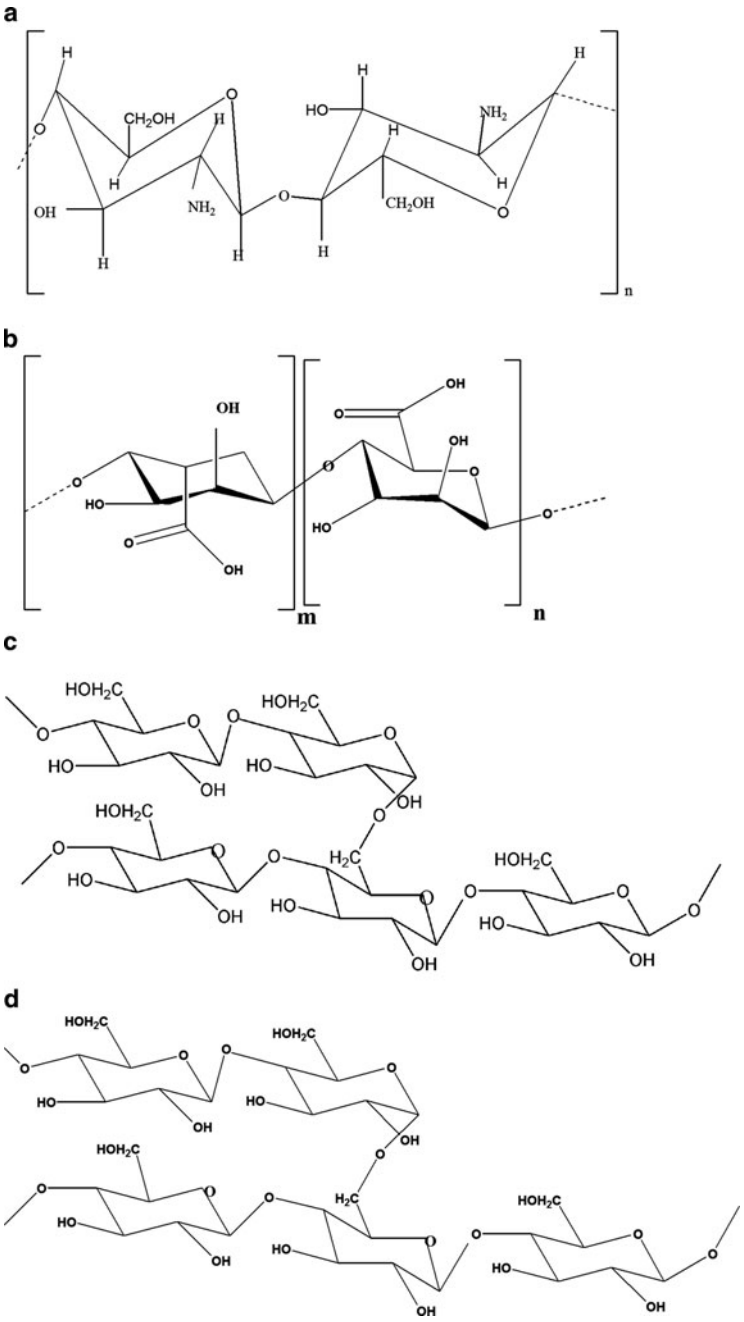

Fig. 1 Natural polymers used in drug delivery: (**a**) chitosan, (**b**) alginate, (**c**) starch, and (**d**) dextran

This mild gelling property allows the encapsulation of various molecules or even cells within alginate gels with minimal negative impact. Furthermore, the carboxylic acid groups of alginic acid are highly reactive and can be appropriately modified for various applications. Alginate has also been extensively investigated as a drug delivery device [18–20] wherein the rate of drug release can be varied by varying the drug polymer interaction as well as by chemically immobilizing the drug to the polymer back bone using the reactive carboxylate groups. Hydrophobically modified alginates are also used for drug delivery applications [21]. The encapsulation of proteins and bioactive factors within ionically crosslinked alginate gels are known to greatly enhance their efficiency and targetability and, as a result, extensive investigation has been undertaken to develop protein delivery systems based on alginate gels [18, 22]. A disadvantage of using alginate-based gels, apart from their poor degradability, is poor cell adhesion on alginate gel. However, the encapsulation efficiency of this system is low, due to its porous nature [23]. The unique property of these linear copolymers is that their surface is negatively charged. When positively charged polymers are added to the alginate solution, they can form a polycation–polyanion complex, which will enhance the overall stability of the microcapsules. Several natural polymers such as chitosan [24] have been used in combination with sodium alginate in order to increase the encapsulation efficiency and hence the protein release profiles. At low pH (gastric environment), alginate shrinks and the encapsulated contents are not released. The payload of, e.g., insulin, could be increased by liposome encapsulation and this lipoinsulin can be entrapped in an alginate system. The aqueous interior of the liposome will preserve the structure and conformation of insulin, while the lipid exterior may help improve absorption across biological barriers. Oral administration of lipoinsulin-loaded alginate–chitosan capsules was found to reduce blood glucose level in diabetic rats. The effect could be observed within 6 h and was prolonged when compared with insulin-loaded alginate–chitosan capsules [25]. It has been reported that purified alginate is nontoxic and biodegradable when given orally.

3.2 Starch

Starch, $(C_6H_{10}O_5)_n$, consists mainly of two glucosidic macromolecules, amylose and amylopectin (Fig. 1c). Due to the substantial swelling and rapid enzymatic degradation of native starch in biological systems, it is not suitable in some controlled drug delivery systems. Starch is a potentially useful polymer for thermoplastic biodegradable materials because of its low cost, availability, and production from renewable resources [26]. However, it has some limitations like low moisture resistance, poor processability (high viscosity), and incompatibility with some hydrophobic polymers. Various strategies like physical or chemical modification of starch granules have been created to overcome these problems [27, 28]. The hydrophilic nature of starch due to the abundance of hydroxyl

groups, is a major constraint that seriously limits the development of starch-based material for industrial applications. Chemical modification has been studied as a way to solve this problem and to produce water-resistant material. Esterification with organic acid is known to result in thermoplastic and hydrophobic starch material.

Mahkam et al. modified chitosan crosslinked starch polymers for oral insulin delivery. Increasing the chitosan content in the copolymer enhanced the hydrolysis in the SIF and thus led to slower release in intestinal pH [29].

3.3 Dextran

Dextran is a polysaccharide consisting of glucose molecules coupled into long branched chains, mainly through the 1,6- and partly through the 1,3-glucosidic linkages (Fig. 1d). Dextrans are colloidal, hydrophilic and water-soluble substances, which are inert in biological systems and do not affect cell viability [30]. Dextrans have been used for many years as blood expanders, whose role is to maintain or replace blood volume and their use as a carrier system for a variety of therapeutic agents have also been investigated, including for antidiabetics, antibiotics, anticancer drugs, peptides and enzyme [31]. Since dextran can be degraded by the enzyme dextranase in the colon, it has been possible to design polymeric prodrugs or nanoparticles for colonic drug delivery based on dextran [32]. Dextran molecules could be degraded by microbial dextranases, which make the ester bond accessible to hydrolysis, thereby releasing the drugs.

Dextran and its derivatives are among the main promising candidates for the preparation of networks capable of giving a sustained release of proteins. Poly (lactide-*co*-glycolide) (PLGA)-grafted dextran was used as a nanoparticulate oral drug carrier. It was expected that dextran would form the hydrophilic outer shell, due to its solubility in water, while PLGA would form the inner core of the nanoparticle, due to its hydrophobic properties. Because the dextran domain can degrade in the colon, nanoparticles of PLGA-grafted dextran can be used as a drug carrier for oral targeting [33].

Sarmento et al. developed nanoparticle insulin delivery system by complexing negatively charged dextran sulfate and positively charged chitosan [34]. The author states that release profile of insulin suggest a dissociation-driven, pH-dependent release mechanism. This was further illustrated by a slower release from particles with a higher ratio of dextran sulfate to chitosan. Increasing the ratio from 1:1 to 2:1 decreased the total insulin release after 24 h from 76 to 59%. Vitamin B12-coated dextran nanoparticle conjugates (150–300 nm) showed profound (70–75% blood glucose reductions) and prolonged (54 h) antidiabetic effects with biphasic behavior in streptozotocin-induced diabetic rats [35]. Modified dextran is also used in gene delivery applications [36, 37].

3.4 Pullulan

Pullulan is a linear bacterial homopolysaccharide formed by glycosidic linkages of α-(1 → 6) D-glucopyranose and α-(1 → 4) D-glucopyranose units in a 1:2 ratio, originating from *Aurebasidium pullulans* [38]. This polysaccharide has numerous uses: in foods and beverages as a filler; in pharmaceuticals as a coating agent; in manufacturing and electronics where it is used because of its film and fiber-forming properties. The backbone structure of pullulan resembles dextran with both of them lending themselves as plasma expanders. Like dextran, pullulan can also be easily derivatized in order to impart new physicochemical properties, e.g., to increase the solubility in organic solvents or to introduce reactive groups.

Anionic and/or amphiphilic pullulan hydrogel microparticles have also been obtained using epichlorohydrin or sodium trimetaphosphate as crosslinking agents [39]. The interactions between lysozyme and various substrates were studied. Such nanoparticles were able to protect the loaded enzyme in acidic environment, thus suggesting its possible use for oral administration of gastro-sensitive drugs. The presence of anionic charges in nanogels crosslinked by trisodium trimetaphosphate also allowed the entrapment of cationic compounds such as methylene blue and polyethylenimine, the model drug's release being affected by the ionic strength of the medium. Cationized pullulan is also used in gene delivery [40].

Though pullulan is not a natural gelling polysaccharide, an appropriate chemical derivatization of its backbone can actually lead to a polymeric system capable of forming hydrogel. Hydrophobized pullulan, i.e., cholesterol-bearing pullulan nanogels were capable of binding various hydrophobic substances and various soluble proteins [41, 42] and has been used as a drug delivery carrier for hydrophobic substances, such as anticancer drugs like adriamycin, doxorubicin etc. and also for gene delivery [43–45].

3.5 Chitosan

Chitosan is a natural, cationic aminopolysaccharide (p*K*a 6.5) copolymer of glucosamine and *N*-acetylglucosamine obtained by the alkaline, partial deacetylation of chitin. It is the second most abundant natural polysaccharide and originates from shells of crustaceans. Chitosan is a biodegradable, biocompatible, positively charged nontoxic mucoadhesive biopolymer. Since chitosan contains primary amino groups in the main backbone that make the surfaces positively charged in biological fluids, biodegradable nano/microparticles can be readily prepared by treating chitosan with a variety of biocompatible polyanionic substances such as sulfate, citrate, and tripolyphosphate [46]. These unique features of chitosan have stimulated development of delivery systems for a wide range of biological agents [47]. Its natural mucoadhesive properties allows design of bioadhesive drug carrier systems that can bind to the intestinal mucosa, and thus improve the residence time

of drugs in the intestinal lumen and, consequently, their bioavailability [48]. Chitosan has been reported to enhance drug permeation across the intestinal, nasal, and buccal mucosa [49]. Chitosan microspheres have arisen as a promising candidate in oral or other mucosal administration for improving the transport of biomacromolecules such as peptides, proteins, oligonucleotides, and plasmids across biological surfaces. This is because chitosan microspheres can improve the drug adsorption via the paracellular route. Chitosan nanoparticles were first prepared in 1997 by Alonso et al. [50] and the same group later used chitosan–insulin nanoparticles for the nasal delivery of insulin in rabbits. Chitosan nanoparticles have been developed to encapsulate proteins and nucleic acids [51]. Chitosan considerably enhanced the absorption of peptides such as insulin and calcitonin across the nasal epithelium. Chitosan is generally considered nontoxic and biodegradable, with an oral LD50 in mice of over 16 g/kg [52].

4 Chitosan Sources and Chemical Structure

Chitin is found in the exoskeleton of some anthropods, insects, and some fungi. Commercial sources of chitin are the shell wastes of crab, shrimp, lobster, etc. Chitosan is usually prepared by the deacetylation of chitin. The conditions used for deacetylation will determine the average molecular weight (Mw) and degree of deacetylation (DD). The structure of chitosan is very similar to that of cellulose [made up of β (1-4)-linked D-glucose units], in which there are hydroxyl groups at C-2 positions of glucose rings. Chitosan is a linear copolymer polysaccharide consisting of β (1-4)-linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) units (see Fig. 1a and graphical abstract) The properties, biodegradability, and biological role of chitosan is frequently dependent on the relative proportions of *N*-acetyl-D-glucosamine and D-glucosamine residues. The term chitosan is used to describe a series of polymers of different Mw and DD, defined in terms of the percentage of primary amino groups in the polymer backbone [53]. The DD of typical commercial chitosan is usually between 70 and 95%, and the Mw between 10 and 1,000 kDa.

4.1 Chemical Methodology for the Preparation of Chitosan

Preparation of chitosan involves four steps: deproteinization, demineralization, decoloration, and deacetylation. Deproteinization is carried out by alkaline treatment using 3–5% NaOH (w/v) aqueous solution at room temperature overnight. Other inorganic constituents remaining are removed by treatment with 3–5% aqueous HCl (w/v) solution at room temperature for 5 h. The product is again reacted with 40–45% NaOH solution at 120°C for 4–5 h. This treatment gives the crude sample of chitosan. The crude sample is purified by precipitating the chitosan

from its aqueous acetic acid solution to NaOH and washing with distilled water until neutralized [54].

4.2 Physicochemical and Biological Properties of Chitosan

Chitosan is a semicrystalline polymer that exhibits polymorphism. Chitosan belongs to a series of polymers with different DD and Mw [55], which are the two important physicochemical properties of chitosan. DD is defined as of the percentage of primary amino groups in the polymer backbone. The DD and Mw of chitosan can be altered by changing the reaction conditions during the manufacture of chitosan from chitin (typical commercial chitosan has a DD of 66–95%). Chitosan appears as colorless, odorless flakes. It is readily soluble in aqueous acidic solution. The solubilization occurs through protonation of amino groups on the C-2 position of D-glucosamine residues, whereby polysaccharide is converted into polycation in acidic media. Chitosan has a low solubility at physiological pH of 7.4 as it is a weak base (p*K*a 6.2–7). Adjusting solution pH to approximately 7.5 induces flocculation due to deprotonation and insolubility of the polymer [56]. Higher Mw chitosan of approximately 1,400 kDa demonstrates a stronger level of mucoadhesion than low Mw chitosan of 500–800 kDa, because the former has a higher level of viscosity. The viscosity of chitosan solution increases with an increase in chitosan concentration and DD but with a decrease in solution temperature and pH. It is known to possess a good complexing capacity. Chitosan can also complex with an oppositely charged polymer such as poly(acrylic acid), sodium salt of poly(acrylic acid), carboxymethyl cellulose, xanthan, carrageenan, alginate, pectin etc. The biological properties of chitosan are illustrated in Fig. 2.

4.3 Limitations

Chitosan suffers from low solubility at a physiological pH of 7.4, limiting its use as absorption enhancer in, for example, nasal or peroral delivery systems [57]. Another limitation of chitosan for the preparation of sustained release systems arises from its rapidly adsorbing water and higher swelling degree in aqueous environments, leading to fast drug release [58]. In order to overcome these problems, a number of chemically modified chitosan derivatives have been synthesized.

4.4 Modification of Chitosan

Most chemical modifications of chitosan are performed at the free amino groups of the glucosamine units. There are also reports on modifications of chitosan hydroxyl

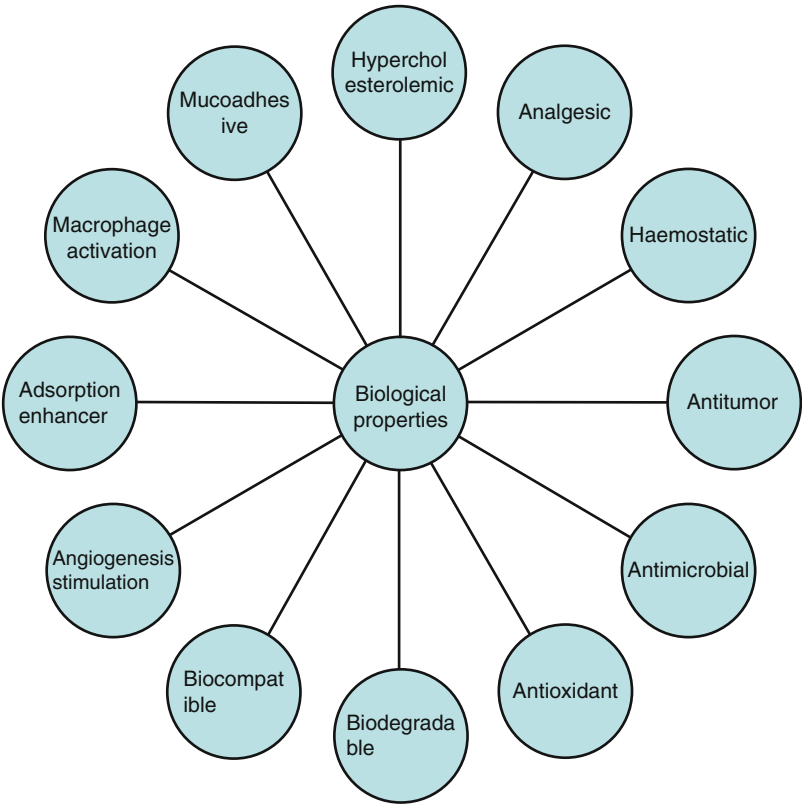

Fig. 2 Biological properties of chitosan

groups [59]. For example, the formation of amide bonds between these amino groups and activated carboxylic groups can be initiated easily. Purification of modified chitosans can often be done by simple dialysis. A great number of chitosan derivatives have been obtained by grafting new functional groups on the chitosan backbone. The chemical modifications (quaternization, acylation, cyclodextrin encapsulation, thiolation etc.) afford a wide range of derivatives with modified properties for specific applications in biomedical and biotechnological fields [60]. Modification does not change the fundamental skeleton of chitosan but brings new or improved properties for, e.g., mucoadhesion and permeation enhancement. The advantage of chitosan over other polysaccharides is that its chemical structure allows specific modifications without too many difficulties at C-2 position. Specific groups can be introduced to design polymers for selected applications. The main reaction easily performed involving the C-2 position is the quaternization of the amino group or a reaction in which an aldehydic function reacts with $–NH_2$ by reductive amination. This latter reaction can be performed in aqueous solution under very mild conditions to obtain randomly distributed substituents in a controlled

amount along the chitosan chain. This method has been proposed for introduction of different functional groups on chitosan using acryl reagents in an aqueous medium; introduction of *N*-cyanoethyl groups is said to produce some crosslinking through a reaction between the nitrile group and the amine group [61]. In addition, it is important to note that more regular and reproducible derivatives should be obtained from highly deacetylated chitin [62]. Assuring control of the quality of the initial material is essential before modification, especially when biological applications are to be explored.

4.4.1 Covalent Modifications

Covalent modification of chitosan includes thiolation and hydrophobic modifications like acylation and quaternization, which will be discussed in later sections (Sections 7.1, 7.2 & 7.3).

4.4.2 Polyelectrolyte Complexes

Polyelectrolyte complex formation occurs when two oppositely charged polymers (polycations and polyanions) in solution phase separate to form a dense polymer phase, known as the coacervate, and a supernatant, which typically has very low concentrations of polymer. A nanoparticle system, composed of hydrophilic chitosan and poly(γ-glutamic) acid (γ-PGA) hydrogels, was prepared by Lin et al. by a simple ionic-gelation method [63]. The prepared nanoparticles with chitosan on the surfaces could efficiently open the tight junctions between Caco-2 cell monolayers. This suggests that these nanoparticles can be an effective intestinal delivery system for peptide and protein drugs and other large hydrophilic molecules. Lin et al. demonstrated that positively charged chitosan PGA nanoparticles (size 110–150 nm) transiently opened the tight junctions between Caco-2 cells and thus increased the paracellular permeability. After loading of insulin, the nanoparticles remained spherical and the insulin release profiles were significantly affected by their stability in distinct pH environments. The in vivo results clearly indicated that the insulin-loaded nanoparticles could effectively reduce the blood glucose level in a diabetic rat model [64].

Sonaje et al. reported the biodistribution of aspart-insulin, a rapid-acting insulin analog, following oral or subcutaneous (SC) administration to rats using a pH-responsive nanoparticle system composed of chitosan and poly(γ-glutamic acid) (CS/γ-PGA NPs). The pharmacodynamic (PD) and pharmacokinetic (PK) evaluation of these nanoparticles in a diabetic rat model produced a slower hypoglycemic response for a prolonged period of time, whereas the SC injection of aspart-insulin produced a more pronounced hypoglycemic effect for a relatively shorter duration. These nanoparticles could effectively adhere on the mucosal surface, increase the

intestinal absorption of insulin, and produce a slower, but prolonged hypoglycemic effect [65].

Transepithelial electrical resistance (TEER) measurements and transport studies implied that CS/γ-PGA NPs can be effective as an insulin carrier only in a limited area of the intestinal lumen where the pH values are close to the p*K*a of chitosan. So, a pH-responsive nanoparticle system was self-assembled by TMC and γ-PGA for oral delivery of insulin. In contrast, TMC(40% Degree of Quaternisation) / γ-PGA NPs may be a suitable carrier for transmucosal delivery of insulin within the entire intestinal tract. The loading efficiency and loading content of insulin in TMC/ γ-PGA NPs were 73.8 ± 2.9% and 23.5 ± 2.1%, respectively. TMC/γ-PGA NPs had superior stability in a broader pH range to CS/γ-PGA NPs; the in vitro release profiles of insulin from both test nanoparticles were significantly affected by their stability at distinct pH environments. TEER experiments showed that TMC/γ-PGA NPs were able to open the tight junctions between Caco-2 cells, and this was further confirmed by confocal microscopy [66].

A biodistribution study in a rat model showed that some of the orally administered CS/γ-PGA NPs were retained in the stomach for a long duration, which might lead to the disintegration of nanoparticles and degradation of insulin [67]. To overcome these problems, nanoparticles were freeze-dried and used to fill an enteric-coated capsule. Upon oral administration, the enteric-coated capsule remained intact in the acidic environment of the stomach, but dissolved rapidly in the proximal segment of the small intestine. In another study, a nanoparticle delivery system self-assembled by the positively charged chitosan and the negatively charged γ-PGA for oral administration of insulin was prepared by mixing the anionic γ-PGA solution with the cationic chitosan solution in presence of $MgSO_4$ and sodium tripolyphosphate [68]. The in vitro results showed that the transport of insulin across Caco-2 cell monolayers by nanoparticles appeared to be pH-dependent; with increasing pH, the amount of insulin transported decreased significantly. The in vivo data indicated that these nanoparticles could effectively adhere on the mucosal surface and their constituted components were able to infiltrate into the mucosal cell membrane. Oral administration of insulin-loaded nanoparticles demonstrated a significant hypoglycemic action for at least 10 h in diabetic rats, and the corresponding relative bioavailability of insulin was found to be 15.1%. Polyelectrolyte complexes of alginate/chitosan nanoparticles were found to be effective for oral insulin delivery [69].

5 Methods for the Preparation of Nano/Microparticles of Chitosan

Various methods for preparation of chitosan nano/microparticles are emulsification/solvent evaporation, spray drying, ionotropic gelation and coacervation, emulsion crosslinking, sieving etc.

5.1 Emulsion Crosslinking

In this method, a water-in-oil emulsion is prepared by emulsifying the chitosan aqueous solution in the oil phase. It is then stabilized by the addition of surfactants. The emulsion thus obtained is crosslinked using crosslinking agents such as glutaraldehyde to harden the droplets. Finally, microspheres are filtered and washed repeatedly with *n*-hexane followed by alcohol, and then dried. The main disadvantage of this method is that the unreacted crosslinking agent cannot be removed completely from the system [70].

5.2 Coacervation/Precipitation

In this method, sodium sulfate solution is added dropwise to an aqueous acidic chitosan soluion containing a surfactant. It is then ultrasonicated for 10 min. Microparticles thus obtained are separated by centrifugation and resuspended in demineralized water. The particles are then crosslinked with glutaraldehyde [71].

5.3 Spray Drying

Drug is first dispersed in an aqueous acidic solution of chitosan. The next step is the addition of a suitable crosslinking agent. This solution is then atomized in a stream of hot air. This leads to the formation of small droplets from which solvent evaporates, leading to the formation of free flowing particles [72].

5.4 Emulsion Droplet Coalescence Method

This method utilizes the principle of emulsion crosslinking and precipitation. In the first step, a stable emulsion containing aqueous solution of chitosan along with the drug is produced in liquid paraffin oil. In the second step, another stable emulsion containing aqueous solution of chitosan in NaOH is prepared. Then both emulsions are mixed under high speed stirring, whereby the droplets of each emulsion collide at random and coalesce, thereby precipitating chitosan droplets to give small-sized particles [73].

5.5 Ionic Gelation

An aqueous acidic solution of chitosan is added dropwise under constant stirring to sodium tripolyphosphate solution. Chitosan undergoes gelation due to the

complexation between oppositely charged species and precipitates to form spherical particles [74].

5.6 Reverse Micellar Method

The surfactant is first dissolved in an organic solvent to produce reverse micelles. To this, an aqueous solution of chitosan and drug are added with constant vortexing to avoid any turbidity. The aqueous solution is kept in such a way as to keep the entire mixture in an optically transparent microemulsion phase. Additional amount of water may be added to obtain nanoparticles of larger size. To this solution, a crosslinking agent is added and the mixture kept overnight under constant stirring. The organic solvent is then evaporated to obtain the transparent dry mass. The material is dispersed in water, followed by the addition of a suitable salt, which helps to precipitates the surfactant out. It is then centrifuged and the supernatant decanted, which contains the drug-loaded nanoparticles. The aqueous dispersion is immediately dialysed through dialysis membrane for about 1 h and the liquid is lyophilized to dry powder [75].

5.7 Seiving Method

An aqueous acidic solution of chitosan is crosslinked using glutaraldehyde. The crosslinked chitosan is then passed through a sieve with a suitable mesh size to obtain microparticles. The microparticles are then washed with 0.1 N sodium hydroxide solution to remove the unreacted glutataraldehyde and dried at 40°C [76].

6 Applications in Drug Delivery

Chitosan is widely used for dental, buccal, gastrointestinal, colon-specific, and gene delivery applications due to its favorable biological properties. It is used in the form of tablets, gels etc. (Fig. 3).

6.1 Drug Delivery for Dental Diseases

Chitosan has been widely used as an effective medicament in various fields of medicine and dentistry. In endodontics, it could be used as an anti-inflammatory root canal dressing material for periapical lesions. Chitosan stimulates the fibroblastic

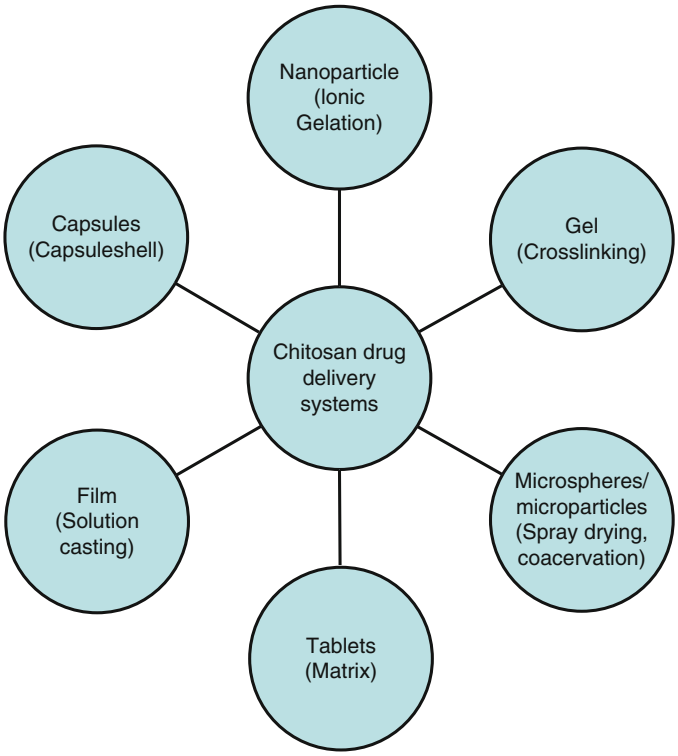

Fig. 3 Different types of chitosan-based drug delivery systems

cells to release chemotactic inflammatory cytokines, especially interleukin 8 (IL-8) [77, 78]. Histological findings indicate that chitosan induces the migration of polymorphonuclear leukocytes and macrophages in the applied tissue at the early stage [79]. At the final stage of wound healing using chitosan, angiogenesis, reorganization of the extracellular matrix, and granulation tissue have been demonstrated [80]. In the case of direct pulp capping as a biological pulp treatment, chitosan produced severe inflammation in the pulp at the early stage [81]. Furthermore, chitosan oligomer (the mixture ranged from dimer to octamer) has been used on the dental pulp tissue and similar initial inflammation was observed. The pulp is surrounded by the hard tissue, dentine. The inflammatory reactions are thought to cause fatal damage to the pulp tissue, and must be overcome before the clinical application of chitosan to dental pulp. D-Glucosamine hydrochloride as a chitosan monomer is easily produced by totally hydrolyzing chitin with hydrochloride, and is the simplest form of chitosan [79]. Chitosan monomer accelerates cell proliferation and differentiation in vitro at a very low concentration, and the ideal tissue regeneration in pulp wounds occurs where there is a minimal initial inflammation reaction [82].

6.2 Buccal Delivery Systems

Drug administration through the buccal mucosa in the mouth provides unique advantages such as avoidance of the hepatic first-pass metabolism, and of the acidity and proteolytic activity of the rest of the gastrointestinal (GI) tract [83]. An ideal buccal delivery system should stay in the oral cavity for few hours and release the drug in a unidirectional way toward the mucosa in a controlled or sustained-release fashion. Mucoadhesive polymers prolong the residence time of the device in the oral cavity whereas bilayered devices ensure that drug release occurs in a unidirectional way [84]. Buccal delivery can be used to treat a number of diseases, such as periodontal disease, stomatitis, fungal and viral infections, and oral cavity cancers. Chitosan is an excellent polymer to be used for buccal delivery because it has muco/bioadhesive properties and it can act as an absorption enhancer [85]. Directly compressible bioadhesive tablets of ketoprofen containing chitosan and sodium alginate in the weight ratio of 1:4 showed sustained release 3 h after intraoral (into sublingual site of rabbits) drug administration [86]. Buccal tablets based on chitosan microspheres containing chlorhexidine diacetate give prolonged release of the drug in the buccal cavity, thus improving the antimicrobial activity of the drug. Chitosan solutions have been evaluated for buccal delivery of proteins; but in order to prolong drug release, films or hydrogels would be more suitable. Chitosan films should degrade slowly under physiological conditions, and for this reason they need to be crosslinked with crosslinking agents like tripolyphosphate. Chitosan hydrogels and films were able to limit adhesion of the common pathogen *Candida albicans* to human buccal cells. These drug delivery systems were also able to sustain drug release (chlorhexidine gluconate) from a hydrogel as well as from film formulations [87]. Chitosan hydrogels were also able to deliver ipriflavone, a lipophilic drug that promotes bone density, into the periodontal pockets. For this purpose, mono- and multilayer composite systems consisting of chitosan and PLGA were designed, and were shown to prolong drug release for 20 days in vitro. Chitosan integrated into bilayered films and tablets with the oral drugs nifedipine and propranolol hydrochloride showed effective buccal membrane adhesion. These complexes were used with and without polyelectrolyte complex (PEC)-forming polymers, such as polycarbophil, sodium alginate, and gellan gum [88].

6.3 Gastrointestinal Delivery

Gastroretentive drug delivery systems increase the retention of a per-oral dosage form in the stomach and offer numerous advantages for drugs exhibiting an absorption window in the GI tract, drugs that are poorly soluble in the alkaline medium (Verapamil), and drugs that are intended for local action on the gastroduodenal wall [89]. Chitosan has a high potential in the development of a successful gastroretentive drug delivery system because it combines both bioadhesion and

floating capabilities [90], especially for drugs that are poorly soluble in intestinal medium and readily soluble in acidic medium. Chitosan could be ideal for use in formulations intended to release drugs slowly in the stomach, since the gel formation by cationic chitosan that is pronounced at acidic pH levels results in marked retardant effects on drug release. Orally administered formulations are initially exposed to the acidic milieu of the stomach, especially if they have been administered to subjects in fasted states, in whom gastric pH is likely to range from approximately 1 to 2. Mucoadhesive ability could result in formulations containing chitosan being retained in the stomach. Adhesion would be expected to be particularly marked under the acidic conditions in the stomach, where cationic chitosan would be highly charged. The chitosan beads can serve as depot reservoir that allows the continuous gradual release of small amounts of Verapamil in solution to the upper part of the small intestine (the main site of absorption), leading to higher and more uniform blood levels of the drug. Thus, reduced adverse effects are highly expected. Floating hollow microcapsules of melatonin showed gastroretentive controlled-release delivery. Release of the drug from these microcapsules is greatly retarded, with release lasting for 1.75–6.7 h in simulated gastric fluid. Most of the mucoadhesive microcapsules are retained in the stomach for more than 10 h (e.g., Metoclopramide- and glipizide-loaded chitosan microspheres) [91].

6.4 Colon-Specific Drug Delivery

Due to its high solubility in gastric fluids, chitosan is widely used for colon-specific drug delivery. Similarly to other polysaccharides, it shows degradation in the colon. Although chitosan can be insoluble in acidic fluids through chemical crosslinking of the microsphere with aldehydes, it is not effective in preventing the release of the encapsulated drugs. To overcome this problem, microencapsulated chitosan microspheres coated with enteric coating materials were developed [92]. The potential of this microsphere was evaluated using sodium diclofenac, an anti-inflammatory drug. Sodium diclofenac was entrapped into the chitosan cores by the spray drying method, after which the chitosan cores were microencapsulated into Eudragit L-100 and Eudragit S-100 using an oil-in-oil solvent evaporation method. The in vitro release studies revealed that no sodium diclofenac was released at gastric pH; however, when the microspheres reached the colonic environment, a continuous release was observed for a variable time (8–12 h). Eudragit S-100-coated chitosan beads developed by Jain et al. exhibited pH-sensitive properties and specific biodegradability for colon-targeted delivery of satranidazole. Eudragit S-100 coating on the chitosan beads prevented premature drug release in simulated upper gastrointestinal conditions and most of the loaded drugs was released in the colon, an environment rich in bacterial enzymes that degrade the chitosan [93]. Chourasia et al. prepared a similar multiparticulate system, by coating crosslinked chitosan microspheres with Eudragit L-100 and S-100 as pH-sensitive polymers, for targeted delivery of the broad-spectrum antibacterial agent metronidazole [94]. The results

showed a pH-dependent release of the drug that was attributable to the presence of Eudragit coating. Moreover, the release of drug was found to be higher in the presence of rat caecal contents, indicating the susceptibility of the chitosan matrix to colonic enzymes. Similar nanoparticular systems for colon-specific delivery of metronidazole were reported by Elzatahry and Eldin [95]. For the treatment of 2,4,6-trinitrobenzene sulfonic acid sodium salt (TNBS)-induced colitis in rats, 5-aminosaliycylic acid (5-ASA) was orally administered using chitosan capsules or a carboxymethyl cellulose (CMC) suspension. Better therapeutic effects were obtained with chitosan capsules than with the CMC suspension. The release of 5-ASA from the chitosan capsule was markedly increased in the presence of rat cecal contents [96]. Degradation of chitosan–tripolyphosphate hydrogel beads in the presence of rat cecal and colonic enzymes indicated the potential of this microparticulate system for colon targeting. The ability of rat cecal and colonic enzymes to degrade chitosan hydrogel beads was independent of pretreatment conditions [97]. Chitosan salts mixed with anti-inflammatory drugs, such as sodium diclofenac, were evaluated for their in vitro release behavior. Chitosan salts, such as glutamic and aspartic salts, provided good controlled release of sodium diclofenac. Slower drug release was obtained with physical mixtures compared to the pure drug both in acidic and alkaline pHs. Interaction with β-glucosidase at pH 7.0 enhanced the release rate [98].

6.5 Mucosal Vaccination

Chitosan is a mucoadhesive polymer that is able to open tight junctions and allow the paracellular transport of molecules across mucosal epithelium, and is therefore suitable for the mucosal delivery of vaccines [99]. Chitosan microparticles were able to associate large amounts of ovalbumin (model vaccine) and diphtheria toxoid (DT). They are not disintegrated in an acidic environment and protect the antigen against degradation by entrapping it into their porous structure [100]. The chitosan microparticles were only taken up by the follicle-associated epithelium (FAE), in which microfold cells (M-cells) are present. This result was from oral delivery studies in mice, in which chitosan microparticles were found to transport associated ovalbumin into the Peyer's patches [101]. Oral efficacy studies with DT associated to chitosan microparticles demonstrated that they were able to induce strong DT-specific systemic and local immune responses (IgG and IgA titers, respectively) [102]. The amount of neutralizing antibodies in mice vaccinated with DT-loaded chitosan microparticles was also high; the levels were considered to be protective in man. Chitosan is shown to be a promising candidate in mucosal vaccine delivery for protein vaccines such as *Bacillus anthracis* [103], diphtheria [104], and influenza [105]. Chitosan derivative trimethylated chitosan/tripolyphosphate/ovalbumin (TMC/TPP/OVA) nanoparticles have previously been shown to be very effective nasal vaccine carriers. Replacing TPP by CpG as a crosslinking agent to obtain TMC/CpG/OVA nanoparticles modulated the immune response towards a Th1

(T helper cell 1) response after nasal vaccination, while maintaining the strong systemic and local antibody responses observed with TMC/TPP nanoparticles. TMC/CpG nanoparticles are therefore an interesting nasal delivery system for vaccines requiring broad humoral as well as strong Th1-type cellular immune responses [106]. In another study, ovalbumin was encapsulated in alginate-coated chitosan nanoparticles and delivered via the oral route to Peyer's patches [107]. Jain et al. [108] observed high secretory IgA concentrations and better immune response with nanoparticulate vesicular formulation upon oral administration. Chitosan microparticles show suitable in vitro and in vivo characteristics for oral vaccination and are therefore a promising carrier system for this particular purpose. Fluorescently labeled chitosan microparticles can be taken up by the epithelium of murine Peyer's patches. Since uptake by Peyer's patches is an essential step in oral vaccination, these results show that the presently developed porous chitosan microparticles are a very promising vaccine delivery system. Chitosan particles have been described as a potential oral vaccine carrier [109] and transport of these particles by M-cells has been observed. A drawback of chitosan is its water solubility. With a pK_a of 6.2, at physiological pH the primary amine groups of chitosan are protonated and, consequently, OVA/chitosan nanoparticles lose their repulsive surface charge and show colloidal instability. The slightly acid environment of the jejunum will promote the stability of chitosan nanoparticles, but as soon as these particles are transported to the subepithelial space to interact with immune cells, the physiological pH will be deleterious for its stability. Because TMC carries a permanent positive charge, OVA/TMC nanoparticles will not be affected by small pH shifts and may be a more suitable carrier for mucosal vaccination [110]. An intraduodenal immunization study with OVA/chitosan nanoparticles, OVA/TMC nanoparticles, and unencapsulated OVA was performed to analyze the extent and type of immune response elicited.

6.6 Gene Delivery

Chitosan is the most prominent among natural polymer-based gene delivery vectors, due to is biodegradability, biocompatibility, and degradation potential [111]. Its role in gene delivery is supported by its ability to protonate in acidic solution and to form a complex with DNA through electrostatic interactions [112]. Mumper et al. first described chitosan as a delivery system for plasmids [113]. Chitosan/DNA nanoparticles may be readily formed by coacervation between the positively charged amine groups on chitosan and negatively charged phosphate groups on DNA. The transfection efficiency of chitosan depends on Mw, DD, pH of the transfecting medium, and cell type. As the Mw increased, transfection efficiency also improved [114]. Moderate DD increases the transfection efficiency. A pH of 6.8–7.0 is found to be optimum to achieve a high level of transfection [115]. Higher gene transfer efficiency was achieved in HEK293 cells as compared to other cell types [116]. But, the major drawback of chitosan is its low transfection efficiency.

Numerous chemical modification like quaternization [117], deoxycholic acid modification [118], galactosylation [119], PEI grafting [120], and thiolation [121] have been carried out by various groups in an attempt to improve transfection efficiency.

7 Chitosan and Its Derivatives

Chemical modification is a powerful tool for controlling the interaction between polymer and drug and thus for enhancing the loading capacity and controlling drug release from the matrix. Modified chitosan improves the bulk properties for the sustained release and has a wide range of pharmaceutical applications.

7.1 Hydrophobic Modification

The hydrophobic character of chitosan can be increased by the covalent attachment of hydrophobic excipients. Hydrophobic interactions are believed to enhance the stability of substituted chitosan by reducing the hydration of the matrix and thereby increasing resistance to degradation by gastric enzymes [122]. Introduction of carboxylic acid groups to chitosan makes chitosan pH-sensitive. Under acidic conditions, the carboxylic groups exist in non-ionized form and are therefore poorly hydrophilic. By contrast, in alkaline conditions, the polymer is ionized and is considerably more hydrophilic. Chitosan succinate with an average diameter of around 50 micrometer, exhibited a loading capacity of around 60% for insulin. Only a small amount of drug was released in simulated gastric fluid, whereas insulin was quickly released in simulated intestinal fluid of pH 7.4. By using chitosan succinate microspheres, the relative pharmacological efficacy was about fourfold improved in comparison to chitosan succinate solution [123]. Chitosan phthalate microspheres (46.34 μm) prepared by emulsion phase separation exhibited a loading capacity of 62%. They protect the insulin from degradation by gastric enzymes [124].

Phthalyl chitosan–poly(ethylene oxide) (PCP) semi-interpenetrating network microparticles developed by Rekha and Sharma showed improved release properties of insulin compared to chitosan [125]. The particle size was about 1.3 μm, with a ζ-potential of about -28.6 ± 12.6 mV and an insulin loading efficiency of 89.6. Insulin release in acidic media was minimized because of the presence of the phthalate group. In another work, Rekha and Sharma tried to establish the role of the hydrophilic/hydrophobic balance on gastrointestinal absorption of peptide drugs along with surface charge of the particles [126]. Lauryl succinyl chitosan particles developed by Rekha and Sharma were found to be highly mucoadhesive, which could be due to the hydrophobic interaction of the lauryl groups with the hydrophobic domains of mucosa, as well as to the negative ζ-potential of the particles. Negatively charged mucoadhesive lauryl succinyl chitosan nano/microparticles (size 315 nm to 1.090 μm) with both hydrophilic (succinyl) and

hydrophobic (lauryl) moieties improved the release characteristics, mucoadhesivity, and the permeability of insulin compared to the native chitosan particles. The presence of hydrophobic moieties controlled the release of the loaded insulin from the particles at intestinal pH. The particles were capable of reducing blood glucose levels in diabetic rats for the duration of about 6 h. The hydrophobic moiety is expected to enhance mucoadhesivity through hydrophobic interactions and also the permeability by loosening the tight junctions.

One of the facile approaches that has drawn much attention is the introduction of hydrophobic groups onto chitosan by acylation (Fig. 4). Fatty acids have been shown to enhance the permeability of peptide drugs [127]. They act primarily on the phospholipid component of the membrane, thereby creating disorder and leading to increased permeability. It has been established by various groups that sodium salts of medium chain fatty acids such as caprylate (C_8), caprate (C_{10}), and laurate (C_{12}) are able to enhance the paracellular permeability of hydrophilic compounds. N-Acylation of chitosan with various fatty acid (C_6–C_{16}) chlorides increased its hydrophobic character and made important changes in its structural features. Tein et al. described the N-acylation of chitosan with fatty acylchlorides (C_8–C_{16}) to introduce hydrophobicity for use as matrix for drug delivery [128]. Anacardoylated chitosan exhibited sustained release of insulin in the intestinal environment, and the released insulin was stable and retained its conformation [129]. The bioadhesive property of chitosan was enhanced by N-acylation with fatty acid chlorides. Chitosan modified with oleoyl chloride showed better mucoadhesion properties than chitosan modified with lower fatty acid groups [130]. Oleoyl chitosan exhibited less swelling than octanoyl chitosan at acidic pH and was capable of maintaining the biological activity of insulin. Compared to octanoyl chitosan, oleoyl chitosan can better resist degradation by gastric enzymes, enhance mucoadhesivity through

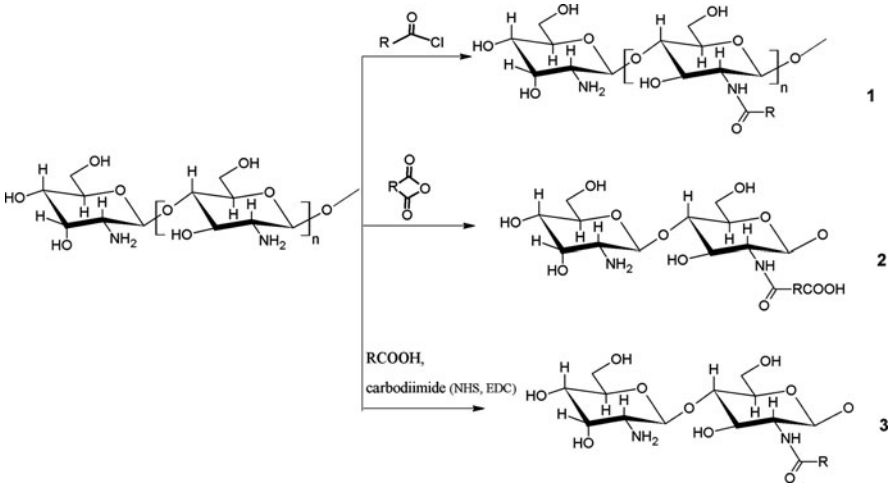

Fig. 4 Acylated chitosan derivatives obtained by the reaction of chitosan with (*1*) acid chloride, (*2*) lactones, and (*3*) carboxylic acids

hydrophobic interactions, and improve permeability by loosening the tight junctions. The oleoyl chitosan derivative exhibited more significant uptake than octanoyl chitosan [131].

7.2 Thiolation

Thiolated polymers/thiomers are hydrophilic macromolecules exhibiting free thiol groups on the polymeric backbone and represent new promise in the field of mucoadhesive polymers. Nowadays, thiolated chitosans are gaining popularity because of their high mucoadhesiveness and extended drug release properties [132]. They are obtained by the derivatization of the primary amino groups of chitosan with coupling reagents. Figure 5 depicts thiolated chitosans obtained by the reaction of chitosan with cysteine, thioglycolic acid, 2-iminothiolane, and thiobutylamidine. The permeation-enhancing effect of thiolated chitosan has been studied with the permeation mediator glutathione, and results indicate that chitosan–TBA/GSH is a potentially valuable tool for inhibiting the ATPase activity of permeability glycoprotein (P-gp) in the intestine [133]. The permeation-enhancing effect also seems to be based on the inhibition of protein tyrosine phosphatase,

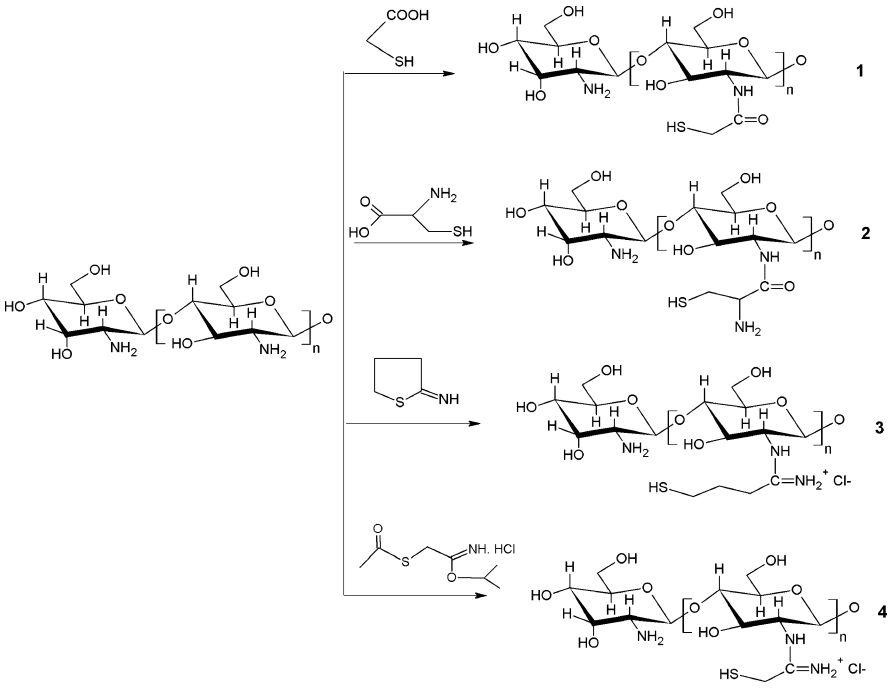

Fig. 5 Thiolated chitosan derivatives obtained by the reaction of chitosan with (*1*) cysteine, (*2*) thioglycolicacid, (*3*) 2-iminothiolane, and (*4*) thiobutylamidine

resulting in an opening of the tight junctions for hydrophilic macromolecules. This theory is supported by various in vitro and in vivo studies in which significantly improved pharmacological efficacy and/or bioavailability of model drugs was seen. Due to the inter- and intramolecular formation of disulfide bonds, a tight three-dimensional network is formed, which leads to high cohesiveness and allows a controlled drug release. Mucoadhesiveness is due to the formation of disulfide bonds with mucus glycoproteins. These thiolated polymers interact with cysteine-rich subdomains of mucus glycoproteins via disulfide exchange reactions. The permeation of paracellular markers through mucosa can be enhanced by utilizing thiolated instead of unmodified chitosan. Moreover, thiolated chitosans display in situ gelling features due to the pH-dependent formation of inter- as well as intramolecular disulfide bonds. This latter process provides strong cohesion and stability of carrier matrices, being based on thiolated chitosans. Moreover, it could be demonstrated that a reversible opening of tight junctions occurs in the presence of thiomers, leading to a more pronounced permeation-enhancing effect of thiomers in comparison to the unmodified polymer. Recently, it has been demonstrated that thiomers are capable of inhibiting efflux pumps such as P-gp, which renders them likely as promising tools for oral delivery of efflux pump substrates.

Trimethyl chitosan–cysteine conjugate (TMC-Cys) was synthesized in an attempt to combine the mucoadhesion and the permeation-enhancing effects of TMC and thiolated polymers related to different mechanisms for oral absorption [134]. TMC-Cys with various Mw (30, 200, and 500 kDa) and quaternization degrees (15 and 30%) was allowed to form polyelectrolyte nanoparticles with insulin through self-assembly, which demonstrated particle size of 100–200 nm, ζ-potential of +12 to +18 mV, and high encapsulation efficiency. TMC-Cys/insulin nanoparticles (TMC-Cys NP) showed a 2.1- to 4.7-fold increase in mucoadhesion compared to TMC/insulin nanoparticles (TMC NP), which might be partly attributed to disulfide bond formation between TMC-Cys and mucin. Insulin solution and TMC NP induced increased insulin transport through rat intestine by 3.3- to 11.7-fold, whereas TMC-Cys NP increased transport by 1.7- to 2.6-fold. Oral and ileal administration of TMC-Cys (200kDa(Mw), 30%(Degree of Quaternization)) NP led to notable hypoglycemic effects as compared to insulin solution, which lasted until 8 h and 7 h post-administration, respectively, with the maximum blood glucose depression of 35 and 70%, respectively.

7.3 Quaternized Chitosan

Quaternized derivatives of chitosan, obtained by introducing various alkyl groups to the amino groups of chitosan molecule structure, were extensively studied for oral insulin delivery [135]. These derivatives are drastically more soluble in neutral and alkaline environments of the intestine and, hence, are more efficient than chitosan for drug delivery and absorption across the intestinal epithelium of the jejunum and ileum. TMC is prepared by reacting chitosan with trimethyliodide

bond. The permeation-enhancing properties of these chitosan derivatives have been attributed to their ionic interactions with the tight junctions and cellular membrane components to increase the paracellular permeation of hydrophilic compounds. The chitosan derivatives in nanoparticle form have less positive surface charge because part of the basic group is involved in the crosslinking reaction with TPP. Their interactions with tight junction are therefore limited and, hence, drug transport across the monolayer more likely occurs through the transcellular pathway rather than by tight junction opening. Recently, nanoparticles based on TMC were proved to interact with intestinal tissue (rat jejunum) and with Caco-2 cell monolayers. In particular, the increase in the quaternization degree of TMC favored mucoadhesion [136]. TMC nanosystems with 35% quaternization combined good penetration-enhancement properties and mucoadhesion. Even if the mucoadhesive properties slowed down the absorption of nanoparticles through the mucus layer into the cell, the increased contact with the intestinal epithelium offered more possibilities for nanoparticle internalization [137]. Chitosan and TMC were demonstrated to be biocompatible both as solutions and in nanoparticulate form. The in vitro model (Caco-2 cells) showed that chitosan and TMC were able to enhance insulin permeation both as nanoparticles and as solutions. Chitosan nanoparticles were still able to widen paracellular pathways, and TMC slightly interfered with substrate integrity. Another quaternized derivative, *N*-hydroxypropyltrimethylammonium chitosan chloride (HTCC), obtained by reacting chitosan with glycidyltrimethylammoniumchloride, was found to be mucoadhesive and was successfully used for oral insulin delivery [138]. Figure 6 depicts two quaternized derivatives of chitosan: TMC and HTCC.

TMC NP was more effective than chitosan nanoparticles and polymer solutions in enhancing permeation across jejunum tissue because the pH of the microclimate mucus environment (pH 6–6.5) was disadvantageous to chitosan and TMC NP has high mucoadhesive potential. Both TMC and chitosan failed penetration enhancement across the ileum, probably due to a thicker mucus layer with barrier properties

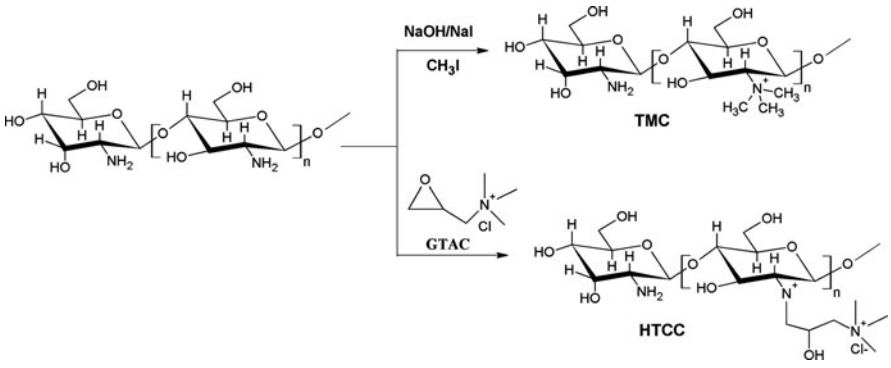

Fig. 6 Quaternized chitosan derivatives: *TMC* trimethylchitosan, HTCC *N*-hydroxypropyltrimethylammoniumchitosan

and a rich enzyme pool. Values for the apparent permeability coefficient (P_{app}) determined by means of an in vitro model (Caco-2) were lower than those observed in ex vivo experiments because the tissue enzymes cause nanoparticle digestion and consequent drug adsorption, and the mucus favors a deep interaction with epithelial cells. The mechanism of penetration enhancement involves the paracellular pathway, with an enlargement of tight junctions for polymer solutions and chitosan nanoparticles, while endocytosis/internalization into duodenum and jejunum epithelial cells was confirmed only for the nanoparticulate form [139].

Jelvehgari et al. investigated the efficiency of nanoparticles formed by a complex coacervation method using chitosan of various Mw and Eudragit L100-55 polymers [140]. As the Mw of chitosan increased, the amount of insulin released increased with respect to time. A new oral delivery system for insulin was developed by Lee et al. aiming to improve bioavailability based on a conjugate between insulin and low Mw chitosans (LMWC; of 3, 6, 9, and 13 kDa) of narrow Mw distribution. LMWC–insulin conjugates after oral administration to diabetic rat models could control blood glucose levels effectively for several hours. Of those conjugates, LMWC(9 kDa)–insulin exhibited the highest pharmacodynamic bioavailability of 3.7 (0.3% relative to that of subcutaneously injected insulin (100%) [141].

7.4 Chemical Grafting of Chitosan

Graft copolymerization onto chitosan is an important field of study for the functionalization and practical use of chitosan [142]. It is an attractive technique for modifying the chemical and physical properties of chitosan. A graft copolymer is a macromolecular chain with one or more species of block connected to the main chain as side chain(s). The properties of the resulting graft copolymers are broadly controlled by the characteristics of the side chains, including molecular structure, length, and number [143]. Grafted chitosan bearing a short graft chain formed a ring structure (40–60 nm in diameter) unimolecularly, that bearing middle chain length was monodisperse spherical (30–40 nm), whereas the longer chain aggregated intermolecularly and led to larger particles (100–400 nm). These studies should be useful for determining a strategy for regulating the molecular design and guest-binding properties of water-soluble grafted chitosan. Grafted chitosans have great utility in controlled release and targeting studies of almost all class of bioactive molecules. The literature suggests that graft copolymerized chitosan is a good candidate in the fields of drug delivery, tissue engineering, antibacterial, biomedical (cardiovascular applications, wound healing), metal adsorption, and dye removal. Grafting can be carried out using free radicals, radiation, or enzymatic and cationic graft copolymerization onto chitosan.

Chitosan graft copolymer nanoparticles (CM, CDM and CTM, based on monomer methyl methacrylate, *N*-dimethylaminoethyl methacrylate hydrochloride, and

N-trimethylaminoethyl methacrylate chloride, respectively) enhanced the absorption and improved the bioavailability of insulin via the GI tract of normal male Sprague-Dawley rats to a greater extent than that of a phosphate-buffered solution of insulin. Increasing the surface charge of the nanoparticles improves insulin encapsulation efficiency and slows the release [144]. The in vivo studies indicate that CDM and CTM nanoparticles seem to be a very promising vehicle for oral administration of hydrophilic proteins and peptides.

8 Conclusion

So far, numerous promising chitosan derivatives exhibiting a better solubility, stronger mucoadhesive capabilities, and enzyme inhibitory properties have been generated. Because of its permeation-enhancing effect, mucoadhesive properties, controlled release properties, and ability to open tight junctions, chitosan is a promising tool for various peptide delivery systems. These properties can be further improved by simple chemical modifications on its primary amino group. These unique features make chitosan and, in particular, its derivatives valuable excipients for drug delivery. Chitosan is still being explored for a variety of applications. The safety of chitosan, its ability to prolong residence time in the GI tract through mucoadhesion, and its ability to enhance absorption by increasing cellular permeability have all been major factors contributing to its widespread evaluation as a component of oral dosage forms.

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