Review Article

Advances in chitosan and Chitosan derivatives for drug delivery perspective

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ABSTRACT

Within the past 25 years, a considerable amount of work has been published on Chitosan and chitosan derivatives and their potential use in drug delivery systems. Being the only cationic polysaccharide, chitosan a member of aminoglucopyran family is being extensively explored for various pharmaceutical and biomedical applications. The cationic character is mainly due to its primary amino groups. The various properties such as controlled drug release, in situ gelation, transfection, mucoadhesion, permeation enhancement, efflux pump inhibitory properties are mainly because of these primary amino groups. On suitable chemical modifications, most of these properties can even be further improved. The In this review, we aim to comprehensively integrate the recent applications of chitosan and its derivatives in oral drug delivery, nasal delivery, colon-specific drug delivery, gene delivery, buccal delivery, ocular drug delivery, vaginal drug delivery, parenteral drug delivery and vaccine delivery.

Keywords: Chitosan, Chitosan derivative, Drug delivery, controlled release.

INTRODUCTION

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 thus helps to improve bioavailability of drugs. Chitosan is the only biodegradable polymer that exhibits a cationic character rendering it unique among all other biodegradable polymers having a monograph inn a pharmacopoeia. The primary amino group gives cationic character to chitosan, responsible for its use in drug delivery systems, and also for its various properties. In the following, these properties are highlighted in more detail.

1. Properties of chitosan

1.1 Controlled drug release

Chitosan is a cationic polymer used for controlled drug delivery. It forms polyion complexes (interpolymer) as a result of its interactions with anionic polymers. The polyion complexes and their basic properties have been investigated for their pharmaceutical application. [1] The specific properties of the complexes (chitosan–sodium alginate and chitosan–sodium acrylate) are due mainly to rigidity or flexibility of the polymer chains. The former is stable to pH change, and the latter is quite sensitive to pH change, which makes them applicable to the design of more precisely controlled drug delivery systems.[2] Anionic polymers helps in achieving controlled release of cationic drugs, however chitosan is the only choice for achieving controlled release of anionic drugs. Polyelectrolyte complexes formed between chitosan derivatives and enoxaparin were very stable and shows significantly improved drug uptake.[3] Bhise et al.[4], for instance, designed sustained release system of anionic drug naproxen and chitosan in complexes prepared by tray drying and spray drying methods. Presence of release retarding polymers in the complex retarded the drug release and improved matrix integrity. Chitosans can also form stable complexes with alginates, carrageenan, pectin, hyaluronic acid. Mainly diffusion and erosion processes are responsible for sustained release from

such complexes.[5] Tripolyphosphate or sulphate can be used as an alternative to anionic polymers.[6]

1.2 In situ gelling properties

Chitosan offers the advantage of in situ gelling properties when its pH-dependant hydratability is addressed properly from the formulation point of view. An in situ gelling delivery system of polyacrylic acid and chitosan was developed. The resulting formulation was in liquid state at pH 6.0 and forms gel at physiological pH of 7.4[7] These in situ gelling properties can even be further improved by thiolation. After having been applied in liquid form, a cross-linking takes place due to access to oxygen on mucosal surfaces resulting in a strong increase in viscosity. Furthermore an even 16500-fold increase in viscosity of an aqueous 1% (m/v) chitosan-thioglycolic acid conjugate was found by utilizing this mechanism.[8]

1.3 Transfection enhancing properties

Stable complexes with chitosan are formed by large polyanionic molecules such as DNA-based drugs and siRNA. Thus, when the ratio of polycationic polymer is sufficiently high nanoparticles can be formed. Because of positive net charge and small size of these particles, endocytosis can be achieved, provided particle size is below 100 nm.[9] Chitosan DNA based drug complex protect to some extent DNA based drugs delivered into the body, resulting in increased bioavailability of the same.[10] Chitosan was modified in order to improve its transfection efficiency. Following different strategies are used for such modification of chitosan. Chitosan/plasmid nanoparticles are stabilized by utilizing thiolated chitosan forming intrachain disulphide bonds within the complex[11]. Malmo et al.[12] investigated the self branching of chitosan as a strategy to improve its gene transfer properties. Results shown that self branched chitosans can yield gene expression levels two and five times higher than those of Lipofectamine and Exgen, respectively. The transfection rate of thiolated chitosan/plasmid nanoparticles was in this connection demonstrated to be five times higher than unmodified chitosan/pDNA nanoparticles. This property could even be further improved by raising the cationic character of thiolated chitosan due to trimethylation of the remaining primary amino groups[13]. PEGylated chitosan and chitosan/cyclodextrin nanoparticles were also promising tools for DNA base drug delivery[14, 15]. Chitosan and lactosylated chitosan carriers were investigated for their transfection efficiencies in vitro[16]. Borchard has recently published a review on the efficient non-viral gene delivery using cationic polymers as DNA-condensing agents.[17] Chitosan is a promising excipient for non-viral gene delivery as it is comparatively less toxic than other cationic polymers such as polyethyleneimine, polylysine, or polyarginine.[18]

1.4 Mucoadhesive properties

The cationic character is mainly responsible for the mucoadhesive properties. The mucus gel layer exhibits anionic substructures in the form of sialic acid and sulfonic acid substructures. Mucoadhesion can be achieved due to the ionic interaction which is present between the cationic primary groups of chitosan and the anionic substructures of the mucus. Furthermore, in order to achieve high mucoadesive a property, the polymer needs to exhibit also high cohesive properties as the adhesive bond otherwise fails within the mucoadhesive polymer rather than between the mucus gel layer and the polymer. In case of chitosans, however, these cohesive properties are also comparatively weak. They can be strongly improved by the formation of complexes with multivalent anionic drugs, multivalent anionic polymeric excipients, and multivalent inorganic anions. When administered with mucoadhesive polymers such as chitosan and Carbomer to rats, the oral bioavailability of buserelin was significantly improved. This effect however could not be observed anymore when chitosan was combined with polyanionic carbomer in the same formulation.[19] Thiolation of chitosan strongly improves its mucoadhesive properties, as the thiolated polymer is capable of forming disulfide bonds with mucus glycoproteins of the

mucus gel layer placing it among the most mucoadhesive polymers known so far.[20] Trimethylation of the primary amino group of chitosan provides an even more cationic character to the polymer. When trimethylated chitosan (TMC) is additionally PEGylated, its mucoadhesive properties are even up to 3.4-fold improved.[21] Mucoadhesive properties of chitosan are comparatively weaker than that of various anionic polymeric excipients such as carbomer, polycarbophil, and hyaluronic acid.[22]

1.5 Permeation enhancing properties

The mechanism being responsible for the permeation enhancing effect of chitosan is also based on the positive charges of the polymer, which seems to interact with the cell membrane resulting in a structural reorganization of tight junction-associated proteins.[23] Schipper et al. [24] could demonstrate that the properties of chitosan such as degree of deacetylation and molecular mass, dictate the permeation enhancing properties, and toxicity to a large extent. Chitosans of high degree of deacetylation, and of high molecular mass exhibit the comparatively highest degree in epithelial permeability. This increase in permeation enhancing effect with increasing molecular mass could also be observed for other permeation enhancing polymers such as polyacrylates.[25] The comparatively high permeation enhancing properties of chitosan seen on Caco-2 cell monolayers, however, are in the presence of mucus layer much lower, as chitosan cannot reach the epithelium because of size limited diffusion and competitive charge interactions with mucins. Nevertheless, this permeation enhancing effect could be confirmed by various in vivo studies. Shah et al., for instance, could demonstrate a 2-fold improved oral bioavailability of ganciclovir due to the co- administration of chitosan. As the polysaccharide acts in a completely different way than most other permeation enhancers, it can be combined with them lading to an additive or even synergistic effect. By using a combination of chitosan and sodium dodecylsulfate, the oral bioavailability of gancilovir was even 4-fold improved, although sodium dodecylsulfate led just to a 2-fold improvement when being co-administered without chitosan.[26] Thiolated chitosan show even more than 30-fold improvement in permeation enhancing properties, on certain mucosal membranes.[27, 28] Trimethylation of the primary amino group however, did lead to further improved permeation enhancing properties.[29] Recently, it was shown that chitosan nanoparticles exhibit only in the first segment of the duodenum a permeation enhancing effect for small peptides, whereas due to the addition of cyclodextrin, this permeation enhancing effect was prolonged over the entire duodenum.[30]

1.6 Colon targeting

Colon specific drug delivery systems have gained increasing attention for the treatment of Crohn's disease, ulcerative colitis and irritable bowel syndrome.[31,32] Chitosan is a well accepted and promising polymer for drug delivery to colonic part, since it can protect therapeutic agents from the hostile conditions of the upper gastrointestinal tract and release the entrapped agent specifically at the colon through degradation of the glycosidic linkages of chitosan by colonic microflora.[33] Nunthanid et al[34, 35] developed spray dried chitosan acetate and ethylcellulose as new compression coats for colon targeted tablets of 5-aminosalicylic acid. Hiorth et al[36] developed pellets with calcium and chitosan in the core. Using interfacial complexation reaction they coated the cores with pectin or alginate. Results shown that the drug release was slowed down compared to uncoated cores. Nanoparticulate system for colon specific delivery of metronidazole was reported by Elzatahry and Eldin.[37] For the treatment of 2, 4, 6-trinitrobenzene sulfonic acid sodium salt (TNBS)-induced colitis in rats, 5-aminosalicylic acid (5-ASA) was orally administered using chitosan capsules or a carboxymethylcellulose (CMC) suspension. Better therapeutic effects were obtained with Chitosan capsules than with the CMC suspension. The release of 5-ASA from the chitosan

capsules was markedly increased in the presence of rat cecal contents indicating the susceptibility of chitosan matrix to colonic enzymes.[31] Chitosan has been used for the specific delivery of insulin to the colon.[38] Varshosaz et al. prepared and evaluated chitosan microspheres coated with cellulose acetate butyrate. These microspheres were prepared by emulsion-solvent evaporation technique, for delivery of 5-ASA into the colon.[39] Chitosan has often been limited in colonic targeting of drugs because of its high solubility in gastric fluids, sometimes resulting in burst release of the drug at the stomach.[40]

1.7 Efflux pump inhibitory properties

In 2002 Carreno-Gomez and Duncan demonstrated efflux pump inhibitory properties for various polysaccharides.[41] Although they did not evaluate chitosan in their studies, it was quite obvious that this polysaccharide will exhibit the same effect. A proof-of-principle for this theory was then provided by Foger et al,[42] demonstrating a significantly improved oral uptake of the P-gp substrate rhodamine 123 due to the co-administration of (thiolated) chitosan in rats. Although this was not a pronounced effect when compared with other efflux pump inhibitors, it seems, nevertheless, useful to improve the mucosal uptake of various efflux pump substrate drugs. Palmberger et al.[43] developed a novel oral delivery system for the efflux pump substrate acyclovir using thiolated chitosan, capable of inhibiting P-glycoprotein (P-gp). The higher the molecular mass of chitosan-4-thiobutylamidine was the more sustained the release of acyclovir.

2. Chitosan drug delivery systems

The above mentioned properties of chitosan are suitable for the development of various drug delivery systems. This can be described as follows:

Oral drug delivery

For controlled release drug delivery systems the oral route is more popular. This is mainly because of greater flexibility in dosage form design when compared with the parenteral route. This is the route having high patient acceptance. In comparison with parenteral dosage form, it is relatively safe route. Also, the constraints of sterility and potential damage at the site of administration are minimal than parenteral forms of administration.[44] The drug thereby can be homogenized with chitosan and directly compressed to tablet. Chitosan loses its mucoadhesive and permeation enhancing properties in distal segments of the intestine, because it precipitates at pH above 6.5. This reduces its applicability to drugs having their absorption window in the proximal segment of the GI tract. The oral bioavailability of acyclovir improved 3-foldand 4-fold due to the incorporation of acyclovir in chitosan and thiolated chitosan, respectively. Within this study, a prolonged residence time in particular of thiolated chitosan microparticles in duodenal and jejunum regions are observed. These data need to be confirmed in human volunteers.[45] Akiyama et al.,[46] for instance, shows an improved bioavailability of various model drugs in human volunteers for mucoadesive formulations which is likely because of an intimate contact of the delivery system with the absorption membrane and a prolonged mucosal residence time of the delivery systems. Chitosan derivatives with comparatively higher cationic character such as trimethylated chitosans,[47] chitosan-thioethylamidine[48] or chitosan-thiobutylamidine conjugates,[49] however do not precipitate anymore within the whole pH range of the GI-tract. Thanou et al.[50] demonstrated that intraduodenally applied buserelin results in 0.8 % absolute bioavailability in rats, whereas co-administrations with trimethylated chitosans resulted in average bioavailability values between 6-13%. In contrast, chitosan HCl did not significantly increase the intestinal absorption at pH 7.2. The adhesion of chitosan granules to the distal esophageal mucosa for 2 hours was demonstrated by Sakkinen et al.[51] In case of nanoparticulate delivery systems, chitosan mostly ionically cross-linked. Precipitation at higher pH is therefore not anymore an issue, as the polymer is already co-precipitated.

Nasal drug delivery

Chitosan has been reported to enhance drug permeation across the intestinal, buccal nasal mucosa.[52] It was found that chitosan microspheres can improve the drug adsorption via the paracellular route. Thus it helps in improving the transport of biomacromolecules such as peptides, proteins, oligonucleotides across biological surfaces. Fernandez- urusuno et al. a, b [53, 54] demonstrated that chitosan nanoparticles (300-400 nm) increase nasal absorption to a large extent than chitosan solutions, mainly because of the intensified contact of the nanoparticles at the nasal mucosa compared to chitosan solutions. Chitosan microspheres and solutions have also been tested for their nasal clearance characteristics in human volunteers using Ƴ-scintigraphy.[55] Bordetella pertussis filamentous haemagglutinin and recombinant pertussis toxin have shown to induce very strong systemic and mucosal immune reactions against the antigens when nasally co-administered with chitosan.[56] Chitosans have been found to increase nasal absorption of D-Arg2-kyotorphin in rats,[57] morphine-6-glucuronide and goserelin in sheep,[58] calcitonin and insulin in rats and sheep.[59] Chitosan particles or polyelectrolyte complexes have been studied for nasal delivery of proteins and peptides.[60,61,62] Fischer et al.[63] developed fentanyl nasal spray formulations with pectin, chitosan, and chitosan-poloxamer 188 for clinical evaluation to provide rapid absorption and subsequently increased bioavailability. The study was conducted in 18 healthy adult volunteers and revealed significantly increased systemic exposure as well as reduced times to peak plasma values for all formulations when compared with oral transmucosal fentanyl citrate lozenge. Significantly improved nasal uptake of isosorbide dinitrate due to the co-administration of chitosan in rat was found in the study of Na et al.[64] Study also showed a minor cilio-inhibiting effect of the polymer.

Buccal drug delivery

Drug administration through the buccal mucosa in the mouth provides unique advantages. The first pass metabolism is avoided also the acidity and proteolytic activity of the rest of the GI tract is avoided, making it an alternative choice to deliver drugs to deliver drugs to the application site.[65] An ideal buccal delivery system should remain in the oral cavity for hours and release the drug in a unidirectional way toward the mucosa in a controlled way. Mucoadhesive polymers prolong the residence time of the drug in the oral cavity whereas bilayered devices ensure that drug release occurs in a unidirectional way.[66] Based on its mucoadhesive as well as absorption enhancement properties, chitosan is an excellent polymer to be used for buccal delivery.[67] Chitosan hydrogels and films were able to limit adhesion of common pathogen Candida albicans to human buccal cells. These drug delivery systems were therefore able to sustain drug release (chlorhexidine gluconate) from a hydrogel as well as from film formulations.[68] Chitosan hydrogels were able to deliver lipophilic drug ipriflavone that promotes bone density. Chitosan integrated in bilayered films and tablets with nifedipine and Propranolol shows effective buccal membrane adhesion. These complexes were used with and without polyelectrolyte complexes (PEC)-forming polymers, such as polycarbophil, sodium alginate, and gellan gum.[69] Directly compressible bioadhesive tablets of ketoprofen containing chitosan and sodium alginate in the weight ratio of 1:4 showed sustained release 3 hr. after intraoral (into sublingual site of rabbits) drug administration.[70] Langoth et al.[71] investigated the potential of the thiolated chitosan for peptide delivery systems via the buccal mucosa in pigs. Thus buccal delivery found useful to treat a number of diseases, such as peritoneal disease, stomatitis, fungal and viral infections, and oral cavity cancers.

Ocular drug delivery

Chitosan is a suitable material for the ocular drug delivery mainly because of its nontoxic character, permeation enhancing properties, and physicochemical characteristics. Chitosan-based formulations used for ophthalmic drug

delivery are hydrogels,[72] nanoparticles[73] and colloidal systems.[74] De Campos et al.[75] demonstrated the potential of chitosan nanoparticles in improving the delivery of drugs to ocular mucosa. Cyclosporin A was chosen as a model drug. Furthermore, chitosan-based colloidal systems were found to work as transmucosal drug carriers, either facilitating the transport of drugs to the inner eye or their accumulation into the corneal epithelia. The use of chitosan-based colloidal suspensions in vivo showed a significant increase in ocular drug bioavailability.[76] The antibacterial activity of chitosan is an advantage for ocular drug administration. Although the precise mechanism of antimicrobial action of chitosan are yet to be elucidated.[77] Felt et al.[78] proposed the use of a chitosan solution as an artificial tear formulation for the treatment of dry eye. Klang et al.[79] described the potential of cationic submicron emulsions for the ocular application of the anti-inflammatory drug piroxicam. Fuente et al.[80] reported ocular delivery of nucleic acids, which mainly consists of hybrid nanostructure of chitosan and the natural occurring glycosaminoglycan hyaluronic acid. Apart from this, DNA-loaded chitosan nanoparticles have unsuccessfully been employed for the transfection of the retina and retinal pigment epithelium.[81]

Vaginal drug delivery

El-Kamel et al.[82] prepared chitosan based vaginal tablets containing metronidazole by directly compressing the polymer, loosely cross-linked with glutaraldehyde, together with sodium alginate with or without microcrystalline cellulose (MCC). They added sodium caboxymethylcellulose (CMC) to some of the formulations. The batch containing 6% chitosan, 24% sodium alginate, 30% sodium CMC and 20% MCC shows adequate release properties in both media and gave lower values of swelling index compared with the other examined formulations. It also proved good adhesion properties with minimum applied weights. Its release properties (% dissolution efficiency, DE) in buffer pH4.8, as well as release mechanism (n values) were found to be negligibly affected by aging. Thus, this formula may be considered a good candidate for vaginal mucoadhesive dosage forms. Sandri et al.[83] evaluated the mucoadhesive and permeation enhancing properties of four different chitosan derivatives: 5-methyl-pyrrolidinone chitosan, two low molecular mass chitosans, a partially re-acetylated chitosan via the vaginal and buccal mucosa by using model drug acyclovir. Methyl-pyrrolidinone chitosan showed the highest mucoadhesive and permeation properties in both vaginal and buccal environments. The capability of enhancing the permeation/penetration of acyclovir was decreased by partial depolymerization of chitosan and disappeared after partial re-acetylation. The antimicrobial properties of chitosan, however, might have a negative impact on the vaginal microflora.[84] Thus, the use of chitosan in antimicrobial system should be based on sufficient knowledge of the complex mechanisms of its antimicrobial mode of action and its vaginal delivery for treating chronic diseases has therefore to be seen with caution.

Parenteral drug delivery

As highly purified chitosan fractions was found neither toxic nor hemolytic, that they have the ability to complex DNA and nuclease degradation and that low molecular weight chitosan can be administered intravenously without liver accumulation suggest there is potential to investigate further low molecular weight chitosans as components of a synthetic gene delivery system.[85] Nordtveit et al.[86] observed that the initial degradation rates of chitosan with lysozyme increased strongly with increasing fraction of acetylated units, this susceptibility of chitosan to lysozyme makes it biodegradable. In controlled release technology, biodegradable polymeric carriers offer potential advantages for prolonged release of low-molecular–weight compounds to macromolecular drugs.[87,88] Onishi et al.[89] performed pharmacokinetic and tissue-distribution studies in mice using fluorescent glycol-chitosan and N-succinyl-chitosan. Both chitosans demonstrated a good retention in blood circulation and a slight

accumulation in tissues, suggesting that chitosan is an effective carrier for drugs that are excreted rapidly. Kim et al.[90] synthesized hydrophobically modified glycol chitosan with 5-β-cholanic acid with incorporated RGD peptide (Arg-Gly-Asp). Intratumoral administration of RG-loaded chitosan nanoparticles demonstrated a substantially decreased tumor growth as compared to the RGD peptide intravenously.

Intravesicle drug delivery

Barthelmes et al.[91] investigated mucoadhesive properties of chitosan and thiolated chitosan nanoparticles on the intravesicle mucosa in order to prolong the residence time of instilled drugs in urinary bladders. The mucoadhesive properties of chitosan thioglycolic acid nanoparticles were determined in porcine urinary bladders and the release of tripolyphosphate among simulated conditions with artificial urine was evaluated. It was shown that thiolated chitosan nanoparticles might be a useful tool for local intravesicle drug delivery, which increases the residence time of the drug and enables sustainable delivery for an extended period of time.

Vaccine delivery

Although chitosan was already shown in the early 1990s to stimulate IgM production of human lymphocytes in serum-free culture.[92] Jabbal-Gill et al.[93] immunised mice by administering intranasally a mixture of Bordetella pertussis filamentous haemagglutinin (FHA) and recombinant pertussis toxin, PT-9K/129G (rPT) in combination with chitosan. For both antigens, this formulation induced systemic responses as measured by serum IgG and also mucosal responses as measured by secretory IgA in lung lavage and nasal washes. The study demonstrated that chitosan potentiated the serum and mucosal immune responses to nasally administered FHA and rPT in mice, showing potential of this nasal chitosan delivery system as a new non-injectable vaccine for the prophylaxis of whooping cough. The potential use of chitosan as a delivery system for inactivated influenza vaccines given intranasally has been clearly demonstrated in mice.[94-96] Read et al.[97] investigated use of chitosan as a nasal delivery system with inactivated, subunit influenza virus. The data show that immunization with chitosan plus trivalent inactivated influenza is a potentially effective, easily-administered form of a vaccination. Lubben et al.[98] prepared and characterized chitosan microparticles. Initial in vivo studies demonstrated that fluorescently labeled chitosan microparticles can be taken up by the epithelium of the murine peyer's patches. Since uptake by murine peyer's patches is an essential step in oral vaccination, these results show that the developed porous chitosan microparticles are a very promising vaccine delivery system. Vila et al. [99] investigated the ability of low molecular weight chitosan in the form of nanoparticles as new long-term nasal vaccine delivery vehicle. Trimethylated chitosan nanoparticles for nasal administration led even to an increased immune response that could be improved by the incorporation of certain immunopotentiators.[100] In case of oral delivery trimethylated-chitosan exhibiting in contrast to unmodified chitosan, an intrinsic adjuvant effect on dentritic cells showed comparatively greater potential.[101]

CONCLUSION

Chitosan exhibits in situ gelling, transfection enhancing, mucoadhesive, permeation enhancing and efflux pump inhibitory properties mainly because of its cationic character. These unique features make chitosan and its derivatives valuable excipients for drug delivery. In addition to above mentioned properties chitosan also have ability to open tight junctions, making it a promising tool for various peptide delivery systems. Chitosan is an important, versatile, natural polymer. Chitosan is very popular because of their cheap production costs, biocompatibility and very low toxicity. Chitosans are useful for tumor treatment by means of development of injectables and in situ gelling system. They can act as delivery vehicle for oral and ophthalmic delivery systems. However, studies toward optimization of process parameters and scale up from the laboratory to pilot plant

and then, to production level are yet to be undertaken. Majority of studies carried out so far are only in in vitro conditions. More in vivo studies need to be carried out. There are surely products based on chitosan and its derivatives will enter the market, the only question is of time of its entry. The very first product containing a chitosan derivative (hydroxypropyl-chitosan) has already been registered under the brand name Ciclopoli®. In the near future, chitosan based drug delivery systems will have more commercial status as compared to that in market in the past.

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