

CHITOSANS VERSUS THIOLATED CHITOSANS AS POLYMERS IN MUCOADHESIVE DRUG DELIVERY - A COMPARITIVE STUDY

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ABSTRACT

The bio polymer chitosan is obtained by alkaline deacetylation of chitin, which is one of the most abundant polysaccharides in nature. Chitosan is a polysaccharide consisting of co polymers of glucosamine and N-acetyl glucosamine. Recently it has been shown that polymers with thiol groups provide much higher adhesive properties than polymers generally considered to be mucoadhesive. The enhancement of muco adhesion can be explained by the formation of covalent bonds between the polymer and mucous layer which is stronger than non-covalent bonds. Thiolated chitosans show more advantages than that of chitosans even though their cost of production is more than that of chitosans. The insitu gel formation within the pH range of 5 to 6.8 makes the application of thiolated chitosans on vaginal, nasal and ocular mucosa also possible.

KEYWORDS: Chitosan, thiolated chitosan, muco adhesion

INTRODUCTION

Muco Adhesive Drug Delivery

Muco adhesion can be defined as the ability of synthetic or biological macro molecule to adhere to the mucosal tissues such as mucosa of small intestine.¹

These muco adhesive drug delivery systems promise several advantages like²:

1. Localisation at a given target site.
2. Prolonged residence time at the site of drug absorption.
3. Intensified contact with the mucosa increasing the drug cone gradient.
4. Bio availability increases.
5. Frequency of dosing decreased resulting in improved patient compliance.

Various natural & synthetic polymers have been discovered as mucoadhesive excipients among which chitosans and thiolated chitosans play significant role.

Chitosans

The bio polymer chitosan is obtained by alkaline deacetylation of chitin, which is one of the most abundant polysaccharide in nature after cellulose¹. Chitin is long and unbranched polymer which is derived from crustaceans. Chitosan is a cationic polysaccharide composed of randomly distributed β (1, 4) linked D-glucosamine (deacetylated unit) and N-acetyl D-glucosamine (acetylated unit). Its β (1, 4) linkage with a 3D helical configuration is stabilised by hydrogen bonding.

Description

There is a growing interest in developing chemical and biochemical processes to obtain & modify bio polymers like chitosans and its derivatives for their applications in different fields of pharmaceutical technology, including the formulation of controlled release dosage forms such as tablets and as permeation enhancing excipient for oral nasal drug delivery and in non-viral gene delivery. Chitin, an abundant polysaccharide is used in the production of chitosan by deacetylation reaction in an alkali

medium. Chitosan offers many advantages and favourable properties, such as enzymatic degradability, non-toxicity, biocompatibility & mucoadhesive properties because of which it has received considerable attention as a novel excipient in drug delivery systems. The solubility of chitosans at acidic P^h is due to the protonation of amino groups at this P^h thus promoting solubility which however is not proper at alkaline & neutral P^h . But the mucoadhesive property of chitosan is explained by fact that the positive charges at neutral P^h interact with negative charges of sialic acid residue of mucous leading to adhesion of these biopolymers to biological membranes for slow release of drug. To improve the mucosal (nasal, preoral) delivery of hydrophilic macromolecules such as peptides, proteins and heparins various derivatives of chitosan such as trimethyl chitosan, mono-n-carboxymethyl chitosan, N-sulfochitosan & chitosan-EDTA conjugates are developed which are effective & safe absorption enhancers than unmodified chitosans. Chitosan has a variety of applications in biomedical field including-wound dressings & drug delivery systems. It is used as a stabilising constituent of liposomes as an excipient controlling drug release in oral formulation. Chitosan is capable of opening the tight junctions and can therefore improve the oral uptake of hydrophilic drugs such as peptides. Concerning gene delivery, chitosan offer the advantage that nano particles can be prepared easily by mixing negatively charged DNA/RNA with the cationic chitosan, leading to stable nanoparticles for controlled drug release. This polymer is also used as a potential adjuvant for swellable controlled drug. It exhibits other biological actions such as hypocholesteremic, anti microbial & in wound healing process. Due to its low toxicity and wide application it is used not only for the purpose of delivery of drugs such as anti-inflammatory, peptides etc. It also acts as biologically active agent. Chitosans associate with vaccines thereby enhancing the antigen uptake by mucosal lymphoid tissues, thus including systemic and immune responses against harmful antigens.

Structure: (fig 1)¹

Preparation

Shell fish wastes from food processing (shrimp, crab, squid, and lobster) were taken and it is de calcified in dil. Aqueous HCl solution (3% to 5% HCl W/V at room temperature) and deproteinated in dil. Aqueous NaOH solution (3% to 5% NaOH, 80° c to 900° c for a few hrs. Or room temperature overnight). Then chitin is obtained after decolourisation in 0.5% aqueous $KMnO_4$ and aqueous oxalic acid. Then by de acetylation in hot concentration NaOH solution (40% to 50% W/V NaOH, at 90° c to 120° c for 4 to 5 hrs). The crude chitosan is dissolved in aqueous 2%W/V acetic acid. Then the insoluble material is removed giving a clear supernatant solution, which is neutralised with NaOH solution resulting in a purified sample of chitosan as a white precipitate. Further purification may be necessary to prepare medical and pharmaceutical grade chitosan.

Advantages

1. Chitosan is a biologically safe polymer and prolongs the adhesion time of oral gels & drug release.
2. It inhibits the adhesion of *Candida albicans* to human buccal cells and has anti fungal activity.
3. Serves a no. of purposes, including-coating agent for tablets, gel former, controlled release matrix, muco adhesion and permeation, enhancement to improve oral availability of drug.
4. Due to mucoadhesive properties, it is a valuable tool for non-invasive drug delivery.
5. In production of micro and nano particles for controlled drug release.
6. In tissue engineering.
7. It enhances the paracellular route of absorption which is important for the transport of hydrophilic compounds.

Pharmaceutical Applications

Ophthalmic drug delivery

Buccal and sublingual drug delivery: Chitosan exhibit favourable biological behaviour, such as bio adhesion, permeability enhancing properties, and interesting physico-chemical characteristics, which make it a unique material for the design of ocular drug delivery vehicles. Due to their elastic properties,

chitosan hydrogels offer better acceptability, with respect to solid or semi solid formulation, for ophthalmic delivery, such as suspensions and ointments.⁴

Buccal drug delivery represents a versatile application, not only in the treatment of local infections, such as periodontal diseases, stomatitis but also a topical route for systemic delivery of therapeutic peptides, avoiding first pass metabolism, acidity and protease activity encountered in the gastro intestinal tract⁵.

Periodontal drug delivery

Chitosans increase the residence time of the formulation in the oral cavity. This will enhance the drug penetration, localise the drug for local therapy, target the diseased tissue, and improve efficacy and acceptability. Being a muco adhesive polymer, chitosan is considered a good candidate for oral cavity drug delivery. The anti bacterial activity of chitosan could be due to the electrostatic interactions between the amine group of chitosan and the anionic sites on bacterial cell wall because of carboxylic acid residues and phospholipids³.

Nasal drug delivery

The nasal mucosa presents an ideal site for bio adhesive drug delivery systems. Bioavailability and residence time of the drugs that are administered via the nasal route can be increased by bio adhesive drug delivery systems. Chitosan drug delivery systems, such as microspheres, liposomes, and gels, have been demonstrated to have good bio adhesive characteristics and swell when in contact with the nasal mucosa⁴.

Gastro intestinal drug delivery

Chitosan granules having internal cavities were prepared by deacidification. When added to acidic and neutral media, these granules were immediately buoyant and provided a controlled release of the desired drug. Both chitosan granules and chitosan laminated preparations could be helpful in developing drug delivery systems that will reduce the effect of gastro intestinal transit time³.

Peroral drug delivery

Chitosan is an excellent candidate for the treatment of oral mucositis. Its bio adhesive and antimicrobial properties offer the palliative effects of an occlusive dressing and the potential for delivering drugs, including anti candidal agents. Nifedepine embedded in a chitosan matrix in the form of beads showed prolonged release of drug compared to granules. Perorally given peptide drugs such as insulin, calcitonin and bruselin have been found to have high bioavailability³.

Intestinal drug delivery

A formulation was developed that could bypass the acidity of the stomach and release the loaded drug for long periods in to the intestine by using the bio adhesiveness of polyacrylic acid, alginate, and chitosan.³ Chitosan microcapsules containing the drug Nitro furantoin showed sustained release of the drug.

Colon drug delivery

Chitosan was used in oral drug formulations to provide sustained release of drugs. Recently, it was found that chitosan is degraded by the micro flora that is available in the colon. Chitosan esters, such as chitosan succinate and chitosan phthalate have been used successfully as potential matrices for the colon-specific oral delivery of diclofenac sodium³.

Transdermal drug delivery

Chitosan has good film forming properties. The drug release from the devices are depends on the membrane thickness and cross linking of the film. Chitosan-alginate poly electrolyte complex (PEC) has been prepared insitu in beads and microspheres for potential applications in packaging, controlled release systems, and wound dressings³.

Vaginal drug delivery

Chitosan vaginal tablet containing metronidazole, acriflavine, and other excipients give adequate release and good adhesion properties³. Chitosan, modified by the introduction of thio glycolic acid to the primary amino groups of the polymer, embeds clotrimazole, an imidazole derivative widely used for the treatment of mycolic infections of the genitourinary tract⁶.

Gene delivery

The course of many hereditary diseases could be reversed by gene delivery. Gene delivery systems include viral vectors, cationic liposomes, polycationic complexes, and micro encapsulated systems. Viral

vector are advantageous for gene delivery because they are highly efficient and have a wide range of cell targets³.

Vaccine delivery

Bovine serum albumin and diphtheria toxoid loaded chitosan microspheres give good tissue compatibility with a long lasting drug delivery system in rats for several days. Various chitosan-antigen nasal vaccines have been prepared. These include cholera toxin, diphtheria toxoid, liposomes, nano particles, attenuated virus and cells, and proteosomes³

Miscellaneous applications

Chitosan membranes surface by complexation and interpenetration of anionic polysaccharides, heparin and dextran sulphate for improved blood compatibility in haemo dialysis. The permeability of urea and creatinine did not change significantly upon modification with heparin and dextran sulphate³.

INDUSTRIAL USES OF CHITOSANS

- Waste Water Purification
- Stabilizing Oil Spills
- Stabilizing Fats in Food Preparation
- Antibacterial Protection for Seeds
- Flavor Stabilizer⁷
- Stabilizes Perishable Fruits/Vegetables
- Ion Exchange Media
- Bacterial Immobilizer
- Cosmetic and Shampoo Additive
- Tableting Excipient
- Absorbant for Heavy Metal Removal⁷.

HEALTH AND NUTRITIONAL USES OF CHITOSANS

- Absorbs and Binds Fat⁸
- Promotes Weight Loss
- Reduces LDL Cholesterol⁹
- Boosts HDL Cholesterol
- Promotes Wound Healing
- Antibacterial/Anticandida/Antiviral
- Acts as Antacid
- Inhibits the Formation of Plaque/Tooth Decay
- Helps Control Blood Pressure
- Helps Dental Restoration/Recovery¹⁰
- Helps to Speed Bone Repair
- Improves Calcium Absorption¹⁰
- Reduces Levels of Uric Acid.

MARKETED PRODUCT CONTAINING CHITOSAN: Ref fig (2)¹¹.

THIOLATED CHITOSANS

Definition

Chitosans by chemical reaction with thiol containing compounds yield thiolated chitosans. Thiolated chitosans can be defined as chitosans with a thiol moiety. The derivatization of the primary amino groups of chitosans with coupling reagents bearing thiol functions leads to the formation of thiolated chitosans.

Description

The polymers with thiol groups are known to have much higher adhesive properties than polymers generally considered to be mucoadhesive. The property of mucoadhesion is enhanced by thiolated polymers due to the formation of covalent bonds between polymers & mucus layer. These covalent bonds are stronger than the non-covalent bonds (like hydrogen bonds which are weak) formed by unmodified chitosans. Thiolated chitosans are also known as thiomers and interact with cysteine rich sub domains of mucus glycoproteins by disulfide exchange improved by immobilisation of thiol groups. Due to formation

of disulphide bonds with mucus glycoproteins, mucoadhesiveness is augmented. To date three different thiolated chitosan derivatives have been synthesized: chitosan-thio glycolic acid conjugates, chitosan-cysteine conjugates and chitosan-4-thio-butyl-amidine (chitosan-TBA) conjugates¹³. These derivatives enhance the solubility of chitosan and also improve the mucoadhesive and or permeation enhancing properties. The strong cohesive properties of thiolated polymers make them highly suitable excipients for controlled drug release. These polymers have numerous applications like gelling properties, in gene delivery, in per oral peptide delivery systems.

Structure: Ex: chitosan-thiobutylamidine^{13&1} Ref fig (3)

Preparation

Synthesis of thiolated chitosans:

The primary amino group at the 2-position of the glucosamine subunit of chitosan is the main target for the immobilization of thiol groups. Sulfhydryl bearing agents can be covalently attached to this primary amino group via the formation of amide or amidine bonds. In case of the formation of amide bonds the carboxylic acid group of the ligands cysteine and thioglycolic acid reacts with the primary amino group of chitosan mediated by a water soluble carbodiimide¹.

Ex: chitosan-TGA conjugates¹³

The chemical modification of chitosan was previously described. Chitosans 500 mg was dissolved in 50 ml of 1.0% acetic acid in order to facilitate reaction between TGA and chitosan, 100mg of 1-ethyl-3-(3-dimethyl amino propyl carbodiimidites(EDAC) was dissolved, 30 ml of TGA was added & the pH was adjusted to 5.0 with 3N NaOH. The reaction was stirred and left for 3 hr at room temperature. To eliminate the unbound TGA & to isolate the polymer conjugates the reaction mixture was dialysed against 5M HCl for 5 times over a period of 3 days in the dark, then 2 times against 5M HCl containing 1.0% NaCl to reduce ionic interactions between the cationic polymer & the anionic sulfhydryl compound.

Advantages

1. These thiolated chitosans have numerous advantageous features in comparison to unmodified chitosan, such as significantly improved mucoadhesive and permeation enhancing properties¹.
2. The strong cohesive properties of thiolated chitosans make highly suitable excipients for controlled drug release dosage forms¹.
3. Solutions of thiolated chitosans display insitu gelling properties at physiological p^h values due to the formation of inter & intra molecular disulphide bonds¹².
4. The performance of thiolated chitosan in in vivo studies, providing proof of their applicability in per oral peptide delivery systems¹.

PHARMACEUTICAL APPLICATIONS

Muco adhesive properties

The improved mucoadhesive properties of thiolated chitosans are explained by the formation of covalent bonds between thiol groups of the polymer and cysteine rich sub domains of glycoprotein in the mucus layer. These covalent bonds are supposedly stronger than noncovalent bonds such as ionic interactions of chitosan with anionic substructures of the mucus layer. It was found that there is a positive correlation between the degree of modification with thiol bearing polymers and their adhesive properties¹.

Permeation enhancing effect

The permeation enhancing effect of chitosan can be greatly improved by the immobilization of thiol groups. The uptake of fluorescence-labelled bacitracin, for instance, was improved 1.6 fold utilising 0.5% of chitosan-cysteine conjugate instead of unmodified chitosan. Chitosans enhance the paracellular route of absorption which is important for transport of hydrophilic macromolecular like peptides. Combination of reduced glutathione with thiolated polymers cause opening of tight junctions in mucus membranes these by increasing permeation^{1,4,12}.

Insitu gelling properties

The efficacy of drug administered to the ocular, nasal & vaginal mucosa depends on the rapid release of drug from the site of its action. To increase bioavailability of drugs, the viscosity of drug formulation must be increased which causes slow release of the drug. Insitu gel formation is the base or

criteria for obtaining drug formulations of sufficient viscosity. Due to the oxidation of thiol groups at physiological p^h range 5-6.8 these thiolated polymers exhibit insitu gelling properties resulting in formation of inter& intra molecular disulphide bonds. These seem to be promising new excipients for liquid or semisolid formulations intended for vaginal, nasal &ocular preparations^{6&12}.

FUTURE TRENDS

Production of micro and nano particles

A controlled drug release out of thiolated chitosan micro particles can be achieved because of formation of disulphide bonds with in polymeric network, microparticles do not disintegrate instead they get strongly stabilized¹.

Tissue engineering

Coating of stents

The enhanced bioavailability is achieved if a controlled release of active ingredient of the formulation is provided. Thiolated polymers play an important role in controlled drug release by forming not only disulphide bonds with mucus glycoproteins but they also form not only disulphide bonds with mucus glycoproteins but they also form inter& intra molecular disulphide bonds which increases the stability of drug & its increased residence time at site of action i.e. sustained release¹.

Non invasive peptide delivery

The incorporation of peptide drugs exhibiting a cationic net charge in anionic mucoadhesive polymers on the one hand leads to a strong reduction in the mucoadhesive properties and, on the other hand, may hinder drug release as a result of strong ionic interactions between the therapeutic ingredient and the polymeric network. Consequently, cationic therapeutic peptides or peptidomimetics such as calcitonin or desmopressin need to be embedded in cationic or non-ionic mucoadhesive polymers^{1&12}.

THIOLATED CHITOSANS AS MATRICES FOR CONTROLLED DRUG RELEASE

Chitosan represents primarily due to its muco adhesive properties, a valuable tool for non-invasive drug delivery⁴.thiolated chitosans also display, besides their strong mucoadhesive and permeation enhancing properties, excellent cohesive properties. The reduced thiol functions on the chitosan backbone enable thiolated chitosans not only to form disulphide bonds with mucus glycoproteins, but also to form inter as well as intra molecular disulphide bonds¹. Such a cross linking of the polymeric chains results in a high stability of drug carrier systems based on thiolated chitosans. The cohesion and stability of a drug delivery system over the intended duration of drug liberation is often a substantial requirement for a controlled release¹³. The usefulness of thiolated chitosans as carrier matrices for controlled drug release was demonstrated with model drugs, such as clotrimazole.

COMPARISON BETWEEN THIOLATED CHITOSANS AND CHITOSANS IN MUCOADHESION:cRef fig (4)¹⁴

CONCLUSION

Thiolated chitosans are far more superior in action than chitosans, being synthetically modified, it can be easily synthesized and the yield is also high resulting into a cost effective alternative for chitosans. More research should be carried out by using thiolated chitosans so that the cost of production of drugs can be reduced to some extent.

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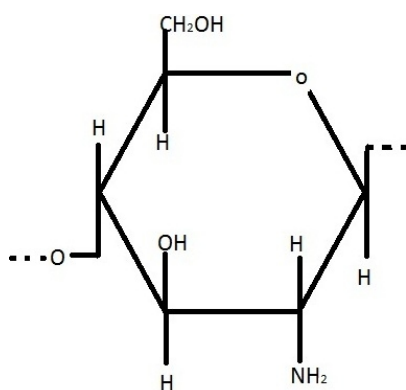


Fig 1: structure of a chitosan



Fig 2: Marketed product containing chitosan

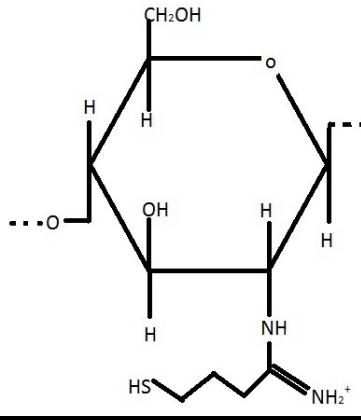
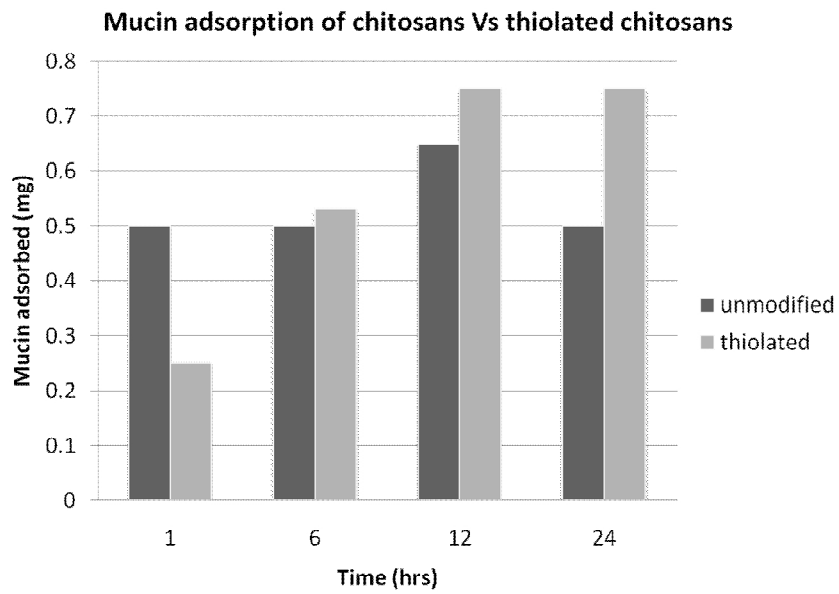


Fig 3: structure of thiolated chitosan (Ex: chitosan-thiobutylamidine)



Adsorption kinetics of mucin with unmodified and thiolated chitosan nano particles

Fig 4: comparison between unmodified and thiolated chitosan in muco adhesion

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