

**THIOLATED CHITOSANS: A NOVEL MUCOADHESIVE POLYMERS: A REVIEW**Vijapur.L.S^{1*}, Sreenivas S.A², Patil.S.H³, Vijapur.P.V⁴, Patwari.P.K⁵ and Saraswathi⁶¹Department of Pharmaceutics, H.S.K.College of Pharmacy, Bagalkot, India²Department of Pharmaceutics, Guru Nanak institute of Pharmacy, Hyderabad, India³Department of Pharmacognosy, Guru Nanak institute of Pharmacy, Hyderabad, India⁴Department of Pharmaceutics, H.S.K.College of Pharmacy, Bagalkot, India⁵Department of Pharmachemistry, Guru Nanak institute of Pharmacy, Hyderabad, India⁶Department of Pharmacology, H.S.K.College of Pharmacy, Bagalkot, India

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ABSTRACT

Chitosan is a natural polycationic copolymer consisting of glucosamine & N-acetylglucosamine units. The polymer has valuable properties as a biomaterial because it is considered to be biocompatible, biodegradable and non-toxic. The purpose of this review article is to provide detailed information about thiolated chitosan which are gaining popularity because of their high mucoadhesiveness and extended drug release properties. The derivatization of the primary amino groups of chitosan with coupling reagents bearing thiol functions leads to the formation of thiolated chitosans or designated as thiomers which possess high mucoadhesion which display thiol bearing side chains. Based on thiol/disulfide exchange reactions and/or a simple oxidation process disulfide bonds are formed between such polymers and cysteine-rich subdomains of mucus glycoprotein's building up the mucus gel layer. Therefore thiomers mimic the natural mechanism of secreted mucus glycoprotein, which are also covalently anchored in the mucus layer by the formation of disulfide bond which is same as bridging structure commonly encountered in biological systems.

Key Words: Chitosan; Thioglycolic acid; Mucoadhesion; Thiomers; Mucus

INTRODUCTION

Drug delivery describes a process whereby a therapeutic agent is administered to the body in a controlled manner. Advanced drug delivery technologies can improve a product clinical and commercial value differentiate a product and serve as an effective resource to outsmart competition. Drug delivery technologies make medicine more convenient and acceptable to a patient by simplifying the dosing regimen and improving administration. Although a number of (NDDS) Novel Drug Delivery System have been successfully commercialized per oral controlled release systems continue to hold the major market share. A significant milestone in NDDS is the development of the Mucoadhesive drug delivery system, an innovative and highly versatile drug delivery system. In the early 80's, Professor Joseph R. Robinson at the University of Wisconsin trail-blazed the concept of mucoadhesion as a new route to prolong the residence time of various drugs on the ocular surface. Over the years mucoadhesive polymers were shown to be able to adhere to the various other mucosal membranes. The capability to adhere to the mucus gel layer which covers epithelial tissues makes such polymers very useful excipients in drug delivery. Mucoadhesive drug delivery systems are delivery systems which utilizes the property of bioadhesion of certain polymers which become adhesive on hydration and hence can be used for targeting a drug to particular region of the body for prolonged period of time^{1,2}.

Since the concept of mucoadhesion has been trail blazed in 1980s, numerous attempts have been made to improve the adhesive properties of polymers. These attempts include approaches such as the use of linear poly(ethylene glycol) as adhesion promoter for hydrogels³, the neutralization of ionic polymers⁴, mucoadhesion by a sustained hydration process³ and the development of polymer-adhesion conjugates^{5,6} providing a specific binding to epithelia. However, all these

systems are based on the formation of non-covalent bonds such as hydrogen bonds, Van der Waal's forces, and ionic interactions. Accordingly, they provide only relative weak mucoadhesion in many cases insufficient for the localization of a delivery system at a given target site. Mucoadhesive polymers in many cases are not proven to be effective as pharmaceutical glue.

The new generation of Mucoadhesive polymers is thiolated polymers popularly known as thiomers. In contrast to well-established Mucoadhesive polymers these novel polymers are capable of forming covalent bonds. The bridging structure most commonly encountered in biological systems the disulfide bond has thereby been discovered for the covalent adhesion of polymers to the mucus gel layer of the mucosa. Thiomers are Mucoadhesive basis of polymers, which display thiol bearing side chain (Fig.I). Based on thiol/disulfide exchange reactions and/or a simple oxidation process as illustrated in (Fig.II) disulfide bonds are formed between such polymers and cysteine-rich subdomains of mucus glycoproteins. Hence, thiomers mimic the natural mechanism of secreted mucus glycoproteins, which are also covalently anchored in the mucus layer by the formation of disulfide bonds.^{7, 8, 9, 10, 11}

THEORIES OF MUCOADHESION

The phenomena of bioadhesion occur by a complex mechanism. Till today, six theories have been proposed which can improve our understanding for the phenomena of adhesion and can also be extended to explain the mechanism of bioadhesion.^{12, 13}

The electronic theory

Theory proposes transfer of electrons amongst the surfaces resulting in the formation of an electrical double layer thereby giving rise to attractive forces.

The wetting theory

Theory postulates that if the contact angle of liquids on the substrate surface is lower, then there is a greater affinity for the liquid to the substrate surfaces.

The cohesive theory

Theory proposes that the phenomena of bioadhesion are mainly due to the intermolecular interactions amongst like-molecules.

The adsorption theory

Theory proposes the presence of intermolecular forces, viz. hydrogen bonding and Vander Waal's forces, for the adhesive interaction amongst the substrate surfaces.

The diffusion theory

Theory assumes the diffusion of the polymer chains present on the substrate surface across the adhesive interface thereby forming a networked structure.

The mechanical theory

Theory explains the diffusion of the liquid adhesives into the micro-cracks and irregularities present on the substrate surface thereby forming an interlocked structure which gives rise to adhesion.

CHITOSAN

The biopolymer chitosan is obtained by alkaline deacetylation of chitin, which is one of the most abundant polysaccharides in nature. Shell wastes of shrimp, lobster and crab are the main industrial sources of chitin. Chitosan is a polysaccharide consisting of copolymers of glucosamine and N-acetyl glucosamine. The primary amino group accounts for the possibility of relatively easy chemical modification of chitosan and salt formation with acids. At acidic pH, the amino groups are protonated which promotes solubility whereas chitosan is insoluble at alkaline and neutral pH 4 because of its favorable properties, such as enzymatic biodegradability, non-toxicity and biocompatibility¹⁴. Chitosan has received considerable attention as a novel excipient in drug delivery systems, and has been included in the European Pharmacopoeia since 2002. So far, Chitosan has been utilized in various fields of pharmaceutical technology including the formulation of controlled release dosage forms such as tablets, gels and microspheres as mucoadhesive and/or permeation enhancing excipient for oral, nasal, ocular and buccal drug delivery^{15, 16} and in non-viral gene delivery.^{17, 18, 19, 20}

THIOLATED CHITOSAN

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 mucoadhesion can be explained by the formation of covalent bonds between the polymer and the mucus layer which are stronger than non-covalent bonds²⁰. To further enhance the solubility of Chitosan and to improve its Mucoadhesive and/or permeation enhancing properties, various derivatives such as trimethylated chitosan²¹, mono-N-carboxymethyl Chitosan²², N-sulfochitosan²³ and chitosan-EDTA conjugates¹⁸ were developed. A further modification is based on the immobilization of thiol bearing moieties on the polymeric backbone of chitosan. To date, three different thiolated chitosan derivatives have been synthesized: chitosan-thioglycolic acid conjugates^{24, 25}, chitosan-cysteine conjugates¹⁸ and chitosan-4-thio-butyl-amidine (chitosan-TBA) conjugates.²⁶

These thiolated chitosans have numerous advantages features in comparison to unmodified chitosan, such as significantly improved mucoadhesive and permeation enhancing

properties^{27, 28}. The strong cohesive properties of thiolated chitosans make them highly suitable excipients for controlled drug release dosage forms^{26, 27}. Moreover, solutions of thiolated chitosans display *in situ* gelling properties at physiological pH values²⁵. It is the aim of this review to provide an overview about different thiolated Chitosan derivatives that have been synthesized so far, as well as their characterization and optimization utilizing various *in vitro* test systems. The performance of thiolated chitosan in *in-vivo* studies, providing proof of their applicability in per oral peptide delivery systems, will be discussed as well.

Thiolation of Chitosan with Thioglycolic acid & Cysteine

The primary amino group at the 2-position of the glucosamine subunit of chitosan is the main target for the immobilization of thiol groups. As shown in Fig.III, sulfhydryl bearing agents can be covalently attached to this primary amino group via the formation of amide or amidine bonds. Thioglycolic acid & Cysteine can be attached covalently to chitosan this was achieved by the formation of amide bonds between the primary amino groups of the polymer and the carboxylic acid group of thioglycolic acid (TGA) & Cysteine. Mediated by a water soluble EDAC in order to activate the carboxylic acid moieties of the subsequently added TGA. The formation of disulfide bonds by air oxidation during synthesis is avoided by performing the process at a pH below 5. At this pH-range the concentration of thiolate anions, representing the reactive form for oxidation of thiol groups, is low, and the formation of disulfide bonds can be almost excluded. Alternatively, the coupling reaction can be performed under inert conditions. In order to remove unbound TGA and to isolate the polymer conjugates, the reaction mixtures were dialyzed in tubing's molecular weight cut-off 12 kDa; using dialysis tubing's, for 3 days at 10°C in the dark against 5mM HCl, then two times against the same medium but containing 1% NaCl to reduce ionic interactions between the cationic polymer and the anionic sulfhydryl compound. Then the samples were dialyzed exhaustively two times against 1mM HCl to adjust the pH of the polymer to 4. Thereafter samples and controls were lyophilized by drying frozen aqueous polymer solutions at 4°C and 0.01 mbar and stored at 4°C²⁹.

Thiolation of Chitosan with 2-iminothiolane/ Traut's reagent

In the case of the formation of amidine bonds, 2-iminothiolane is used as a coupling reagent. It offers the advantage of a simple one step coupling reaction. In addition, the thiol group of the reagent is protected against oxidation because of the chemical structure of the reagent. Thiolated chitosans showed that a degree of modification of 25–250 mmol thiol groups per g chitosan leads to the highest improvement in the mucoadhesive and permeation enhancing properties²⁶.

Thiolation of Chitosan with 4-Mercapto benzoic acid

The covalent attachment of 4-Mercapto benzoic acid (4-MBA) to chitosan was achieved via the formation of amide bonds between the carboxylic acid group of the 4-MBA and amine groups of chitosan mediated by EDAC. The enhanced reactivity of 4-mercaptobenzoic acid in the intestinal pH range should favor the adhesive interactions with the mucus, allowing the thiomers incorporated drug to stay in contact with the absorption site for a prolonged period of time, therefore favoring its uptake. Orientating studies with all these thiolated chitosans showed that a degree of modification of 98.49-176.27 mmol thiol groups per g of chitosan. Due to

this a unique type of thiolated chitosan was synthesized which exhibited its full reactivity at intestinal pH with excellent *in-situ* gelling properties and improved mucoadhesive properties compared to aliphatic thiolated chitosans with similar coupling rates³⁰.

Thiolation of Chitosan with Thioethylamide

Cationic thiomers are exclusively based on chitosan. The primary amino group at the 2-position of the glucosamine subunits of chitosan is the main target for the immobilization of thiol groups. Thiol-bearing compounds can be covalently linked to the primary amino group via the formation of amide or amidine bonds. With appropriate ligand such as Thioethylamide (TEA), In the case of chitosan-4-thio-butyl-amidine (TBA) and Ch-TEA conjugates, they exhibit additionally increased mucoadhesive properties due to improved ionic interactions between cationic amidine substructure of the conjugate and the anionic substructures of the mucus layer. Ch-TEA conjugate can be synthesized by modifying chitosan with isopropyl- S-acetylthioacetimidate hydrochloride (i-PATAI), bearing a protected thiol moiety. The formation of cyclic non-thiol products during synthesis and storage is excluded in contrast to Ch-TBA conjugate where 2- iminothiolane is used as a modifying reagent³¹. The resulting Ch-TEA conjugate exhibited maximum 140 mmol immobilized free thiol groups per gm polymer and showed improved mucoadhesive properties and a good swelling behavior³².

PERMEATION ENHANCING EFFECT

In 1994 Illum et al. showed the permeation enhancing capabilities of chitosan for the first time³³. Chitosan is able to enhance the paracellular route of absorption, which is important for the transport of hydrophilic compounds such as therapeutic peptides and antisense oligonucleotides across the membrane⁶¹. Various studies carried out on Caco-2 cell monolayers demonstrated a significant decrease in the transepithelial electrical resistance after the addition of chitosan^{34, 35}. The mechanism underlying this permeation enhancing effect seems to be based on the positive charges of the polymer, which interact with the cell membrane resulting in a structural reorganization of tight junction-associated proteins³⁶. In the presence of the mucus layer, however, this permeation enhancing effect is comparatively lower, as chitosan cannot reach the epithelium because of size limited diffusion and/or competitive charge interactions with mucins³⁷. Nevertheless, these results obtained on Caco-2 cell monolayer's could be confirmed by *in vivo* studies, showing an enhanced intestinal absorption of the peptide drug buserelin in rats due to the co-administration of chitosan hydrochloride³⁸.

The permeation enhancing effect of chitosan can be strongly improved by the immobilization of thiol group. This effect of thiolated chitosans could meanwhile be shown in various permeation studies in Ussing type chambers using freshly excised intestinal mucosa.

The uptake of fluorescence labeled bacitracin, for instance, was improved 1.6-fold utilizing 0.5% of chitosan-cysteine conjugate instead of unmodified chitosan³⁹. In another study the permeation enhancing effect of chitosan-TBA in comparison to the permeation enhancing effect of unmodified chitosan was shown (Fig.IV). The uptake of the cationic marker compound rhodamine 123 was 3-fold higher in the presence of thiolated chitosan versus unmodified chitosans⁴⁰. The likely mechanism responsible for this improved permeation enhancement has been ascribed to the inhibition

of protein tyrosine phosphates. This enzyme seems to be involved in the opening and closing process of the tight junctions. Protein tyrosine phosphates is responsible for the dephosphorylation of tyrosine subunits of occludin, representing an essential transmembrane protein of the tight junctions. When these tyrosine subunits of occludin are dephosphorylated, the tight junctions are closed. In contrast, when these tyrosine subunits are phosphorylated, the tight junctions are opened.

The inhibition of protein tyrosine phosphates by compounds such as phenylarsine oxide, pervanadate or reduced glutathione leads consequently to a phosphorylation and opening of the tight junctions^{40, 41}. In contrast to the stable but toxic protein tyrosine phosphatase inhibitors phenylarsine oxide and pervanadate, the inhibitory effect of glutathione is lower as it is rapidly oxidized on the cell surface losing its inhibitory activity⁴². Due to the combination of reduced glutathione with thiolated chitosans, however, this oxidation of the inhibitor on the membrane can be restricted, as thiomers are capable of reducing oxidized glutathione⁴³.

THIOLATED CHITOSANS AS MATRICES FOR CONTROLLED DRUG RELEASE

Clotrimazole is well-established as an antimycotic drug in the treatment of vaginal infections. In order to improve its therapeutic efficacy, a sustained release of the drug over a period of several days might be highly beneficial. The modification of chitosan was by using TGA to link with primary amine of chitosan. The amount of EDAC were varied which resulted in Chitosan-TGA conjugates A (70mM) & B (125mM) with 160μM & 280μM thiol groups per g of polymer. The disintegration time of conjugate was prolonged 1.6 fold for conjugate A & 100 fold for conjugate B, Adhesion of Chitosan-TGA-B tablet on vaginal mucosa was 26 times longer than unmodified polymer. When Chitosan-TGA was used for vaginal gel for treating human papilloma Virus (HPV) with clomiphene citrate, the gel adhered to vaginal mucosa for more than 12hrs. Thus Chitosan-TGA is promising vehicle for vaginal application^{44, 47}.

Nasal cavity presents an excellent route for the administration of chitosan based drug delivery systems because of electrostatic interaction between negatively charged mucin & positively charged chitosan⁴⁵. In addition to this chitosan has anti-inflammatory & immunostimulatory activity, & is also able to increase transcellular & paracellular transport across the mucosal epithelium³⁴. Theophylline delivered intranasally as a complex with thiolated chitosan affords protecting against the allergic lung inflammation. The anti-inflammatory effect of theophylline was markedly enhanced when the drug was delivered by Chitosan-TGA compared to unmodified chitosan or theophylline alone. The increased mucoadhesion of Chitosan-TGA provides an effective mucosal drug delivery platform for Nasal & pulmonary drug delivery system⁴⁶.

The retention time of acyclovir at its absorption site, i.e. the upper GIT, was increased by formulating it into microspheres using chitosan, thiolated chitosan, Carbopol 71G or Methocel K15M. The microspheres prepared from thiolated chitosan showed the highest mucoadhesiveness. Further, they were observed to penetrate through the intestinal mucosa qualitatively better than the microspheres prepared from chitosan, Carbopol 71G or Methocel K15M. These properties enabled sustained release of acyclovir from microspheres prepared from thiolated chitosan, and plasma drug concentration in rats was maintained for 24 h. Hence,

microspheres of acyclovir prepared from thiolated chitosan may represent a useful approach for targeting its release at its site of absorption, sustaining its release and improving its oral availability⁴⁸.

Thiolated chitosan were used in the development of polysaccharide-coated nanoparticles in order to confer specific functionality to the system. After chemical modification of commercial and hydrolysed chitosan (400,000 and 9400 g/mol respectively), thiolated chitosans were used to elaborate particles in the nano-range. They were characterized in terms of size and surface charge measurement. Both analyses showed nanoparticles of mean hydrodynamic diameter around 200 nm and positive zeta potential values, indicating the presence of the cationic polysaccharide at the nanoparticle surface. Moreover, the Ellman's reaction was used to demonstrate the presence of thiol groups at the particle surface. The observation of nanoparticles by scanning electronic microscopy (SEM) showed spherical nanoparticles for all formulations. This new system, combining both the advantages of thiolated polymers and colloidal particles can be proposed as a drug carrier system for mucosal delivery of biotechnology products^{49, 60}.

Thiolated polymers, so called thiomers, have been reported to modulate drug absorption by inhibition of intestinal P-glycoprotein (P-gp). Studies were conducted to provide a proof-of-principle for a delivery system based on thiolated chitosan *in-vivo* in rats, using rhodamine-123 (Rho-123) as representative P-gp substrate. *In-vitro*, the permeation enhancing effect of unmodified chitosan, chitosan-4 thiobutylamidine (Ch-TBA) and the combination of Ch-TBA with reduced glutathione (GSH) were evaluated by using freshly excised rat intestinal mucosa mounted in Ussing-type chambers. In comparison to buffer only, Rho-123 transport in presence of 0.5% (w/v) chitosan, 0.5% (w/v) Ch-TBA and the combination of 0.5% (w/v) Ch-TBA/0.5% (w/v) GSH, was 1.8-fold, 2.6-fold, 3.8-fold improved, respectively. Furthermore, enteric-coated tablets based on unmodified chitosan or Ch-TBA/GSH, were investigated *in-vivo*³⁹. In rats, the Ch-TBA/GSH tablets increased the area under the plasma concentration time curve (AUC₀₋₁₂) of Rho-123 by 217% in comparison to buffer control and by 58% in comparison to unmodified chitosan. This *in-vivo* study showed that a delivery system based on thiolated chitosan significantly increased the oral bioavailability of P-gp substrate Rho-123. Within these studies a delivery system based on thiolated chitosan showed a very promising tool for oral administration of P-gp substrates. Besides the Ch-TBA/GSH system other polymers, especially Pluronics have been reported to enhance bioavailability of actively secreted compounds. Multifunctional polymers offer advantages, such as mucoadhesive properties⁵⁰ providing an intimate contact with the area of drug absorption and additionally, systemic side effects might be excluded, as polymers will remain in the gut in large part due to their high molecular weight⁵¹. Subsequent investigations, such as a direct *in-vivo* comparison of different polymers, which have been recently reported to exert an inhibitory effect on efflux proteins, might be useful⁵².

FUTURE TRENDS

Since a 'proof of concept' has already been provided for thiolated chitosans as useful excipients for oral peptide delivery systems, these polymers will certainly be used in various further oral peptide formulations. 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. As non-ionic polymers cannot provide sufficient high mucoadhesion and thiolated chitosans display comparatively the highest Mucoadhesive properties among cationic polymers, this type of thiomers seems to be a favorable tool for the oral administration of cationic hydrophilic macromolecules. Apart from oral delivery systems thiolated chitosans seem to be useful also for other non-invasive routes of peptide drug administration. In particular the nasal, vaginal, buccal and ocular mucosa is interesting targets.

Micro & Nano particles

Microparticles based on chitosan disintegrate very rapidly unless they are combined with multivalent anionic compounds such as sodium sulfate⁵³ or alginate⁵⁴ leading to a stabilization by an ionic cross-linking process. Due to the addition of such multivalent anionic compounds, however, the mucoadhesive properties of chitosan are strongly reduced. In contrast, microparticles that are based on thiolated chitosan do not disintegrate. Because of the formation of disulfide bonds within the polymeric network, microparticles are strongly stabilized⁵⁵. Consequently, a controlled drug release out of thiolated chitosan microparticles can be provided. In contrast to the addition of multivalent anionic compounds, the immobilization of thiol groups on chitosan leads to strongly improved Mucoadhesive properties^{56, 70}.

Coating of stents

Another promising application of thiolated chitosans is their use as coating material for stents. Polymer-coated drug-eluting stents are a potential technique to achieve high local tissue concentrations of an effective drug at the precise site and at the time of vessel injury. First orientating studies demonstrated that by simply dipping the stent in a thiolated chitosan solution and drying it on air, a stable coating could be achieved. During the drying process a cross-linking process of chitosan by the formation of disulfide bonds due to air oxidation takes place. The polymeric network is thereby stabilized on the stent. The chitosan coating should allow sustained release of incorporated drugs, such as anti-inflammatory agents or agents avoiding cell proliferation. Recently, it was shown that stents can be successfully coated with thiolated poly (acrylic acid) and that a sustained release of a model peptide drug out of this thiomeric coating can be provided⁵⁷.

Tissue Engineering

A further interesting application of thiolated chitosans is their use in tissue engineering. The expanding field of tissue engineering applications has accelerated the need for materials which are tissue compatible, biodegradable and with mechanical properties similar to the target tissues. Biodegradable and biocompatible polymers have been attractive candidates for scaffolding materials because they degrade as the new tissues are formed, eventually without inflammatory reactions or toxic degradation⁵⁸. Recently, it has been demonstrated that biodegradability of thiolated chitosan paving the way for its use as novel scaffold material²⁹. Further studies in this direction were performed with L-929 mouse fibroblasts seeded onto chitosan-

thioglycolic acid sheets. Results of this study showed that thiolated chitosan can provide a porous scaffold structure guaranteeing cell anchorage, proliferation and tissue formation in three dimensions⁵¹. Due to the in situ gelling properties it seems possible to provide a certain shape of the scaffold material by pouring a liquid thiolated chitosan cell suspension in a mold. Furthermore, liquid polymer cell suspensions may be applied by injection forming semi-solid scaffolds at the site of tissue damage. Since low concentrated aqueous solutions of thiolated chitosan remain liquid when stored under inert conditions and are rapidly gelling under access of oxygen, they seem to be promising candidates for such applications.

Inhibition of Cytochrome P450

CYP450 enzymes are a family of heme-thiolate proteins with the identity of the fifth ligand to the heme formed by a cysteine residue. In mammals, these enzymes are membrane-bound (usually in the endoplasmic reticulum)^{62, 66}. However, only rudimentary information is available on the exact localization and distribution of CYP450 proteins in the GI tract⁶³. CYP450 enzymes require reducing equivalents, i.e., electrons, from the cofactor NADPH for their catalytic activity. Those electrons are usually provided indirectly via an accessory enzyme, NADPH-P450 reductase that is also a membrane-bound enzyme^{64,65,67}. The proposed mechanism responsible for the inhibition of CYP450 activity across the cell membrane by non-absorbable sulfhydryl compounds like thiomers seems to be based on: (1) the attachment of Mucoadhesive thiomers with mucus layer via formation of disulfide bonds with cysteine-rich subdomains of the mucus layer and (2) formation of disulfide bonds between thiomers and CYP450 enzymes across the semipermeable cell membrane utilizing electrons coming from flavoprotein NADPH-P450 reductase. Thus, unavailability of reducing environment, i.e., electrons on one hand and the formation of disulfide bond on the other hand makes CYP450 enzymes inactive for metabolism. Whether thiomers can be up taken by enterocytes in significant quantities and can subsequently also interact with CYP450 enzymes in the endoplasmic reticulum will strongly depend on their molecular mass and hydrophilic/lipophilic character. Because of high potential to inhibit CYP450s activity, the thiolated polymer conjugates represent a useful polymeric carrier matrix to avoid intestinal metabolism and could be potentially valuable tools for improving the oral bioavailability of various pharmaceutical active ingredients^{68, 69}.

CONCLUSION

The chemical modification of chitosan via derivatization with various reagents bearing sulfhydryl functions causes a dramatic change in the polymer's properties. Mucoadhesiveness and cohesiveness are strongly improved. A comparatively stronger permeation enhancing effect is provided which can be further raised by the combination of thiolated chitosans with the permeation mediator, glutathione. Furthermore, thiolated chitosans display *in-situ* gelling features and facilitate controlled drug release. Due to these advantageous features thiolated chitosans have been successfully used for per oral administration of peptide drugs. They seem to represent a promising new generation of polymeric excipients, in particular for the noninvasive administration of hydrophilic macromolecular drugs. Thiolated chitosans might prove successful, as scaffold material in tissue engineering and as coating material for stents with Inhibition of cytochrome P450. Taking the great

potential of mucoadhesive polymers into consideration, this class of polymers will certainly further alter the landscape of drug delivery and contribute towards the development of more efficient therapeutic systems.

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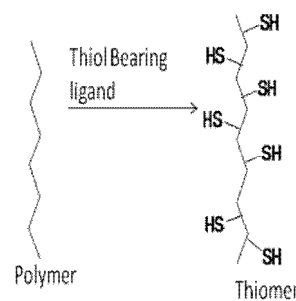


Fig.I Thiomer

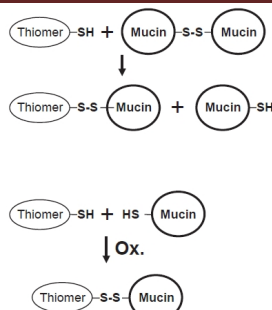


Fig. II Disulfide bond formation between Thiomers with Mucus Glycoprotein

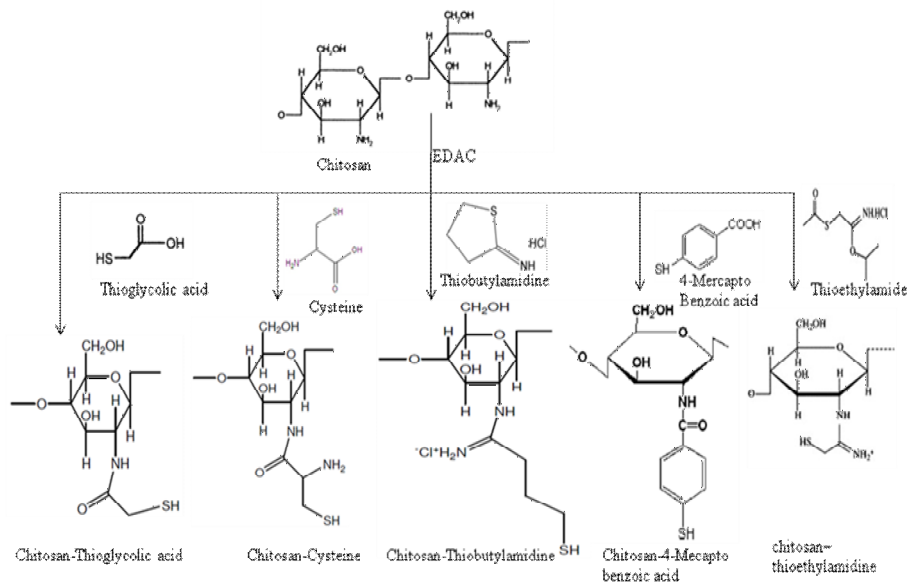


Fig.III Synthetic pathway for the modification of Chitosan with various Thiolating agents

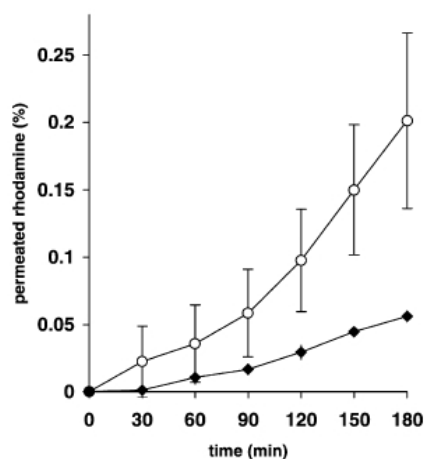


Fig.IV Permeation enhancing effect of 0.5% (m/v) chitosan-TBA conjugate with 5% (m/v) glutathione (W) and 0.5% (m/v) unmodified chitosan (V) on small intestinal mucosa.