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Thiomer- A new generation mucoadhesive

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ABSTRACT

The advantages of new generation mucoadhesive are increased cohesive force, increased enzyme inhibiting property, increased permeation effect of paracellular drug. In new generation mucoadhesive include l-cysteine into thiolated polymer and lectin mediated adhesive polymers. Thiolated polymers are immobilization of sulhydryl-bearing ligands on the polymeric backbone polymers, like chitosan and poly (acrylates). Thiomer micro- and nano-particles exhibit as the delivery of various types of challenging drugs. This achievement leads to significantly improved properties compared with the corresponding unmodified polymers. Thiomer micro and nano particulate delivery systems can be developed by different techniques, such as insitu gelation and covalent crosslinking, radical emulsion polymerization, emulsification and solvent evaporation. Mucoadhesive properties are strongly improved by the development of disulfide bonds between thiol groups of the thiomer and cysteine-rich glycoproteins of the mucus gel layer. Although, enzyme- and efflux-pump inhibiting, improved permeation-enhancing properties, that is advantages of polymer thiolization. As thiomer micro- and nano-particles were shown to exhibit the same features as thiolated polymers per se, they might be useful tools for the delivery of various types of challenging drugs

Keywords: Thiomer, mucoadhesive, thiol group, stability.

INTRODUCTION

Numerous attempts have been taken to improve the adhesive properties of polymers. For improving adhesive property use of linear polymer (ethylene glycol) as adhesion promoter for hydrogels, mucoadhesion by a sustained hydration process and the development of polymer adhesive conjugates providing a specific binding to epithelia. However, all these systems are based on the formation of non-covalent bonds such as hydrogen bonds, vander Waal's forces, and ionic interactions. Accordingly, they provide only relative weak mucoadhesion in many cases not to be developed the localization of a drug delivery system at a given target site.

A new generation of mucoadhesive polymers are thiolated polymers designated thiomers, these novel polymers are efficient to forming covalent bonds, disulfide bond. Thiomers are mucoadhesive basis polymers, which having property thiol bearing side chains. In thiol or disulfide reactions having oxidation process in which disulfide bonds are found between polymers and cysteine rich subdomains of mucus glycoproteins. Hence, thiomers mimic the natural mechanism of secreted mucus glycoproteins, which are covalently attached in the mucus layer by the formation of disulfide bonds.

Classification of thiomers: Thiomers can be classified in different ways. But the major classification of thiomers is as following-

Cationic thiomers: The main target for the immobilization of thiol groups is that amino group at the 2-position of the glucosamine subunits. Sulfhydryl groups can be covalently attached to 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 for instance by carbodiimides. An unintended oxidation of thiol groups during synthesis can be avoided by performing the reaction under inert conditions. Alternatively the synthesis can be performed at a pH < 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 almost be excluded. Furthermore, disulfide bonds can be reduced after the synthesis process by the addition of reducing agents such as dithiothreitol or borohydride.^[1]

Anionic thiomers: So far generated anionic thiomers exhibit all carboxylic acid groups as anionic substructures. These carboxylic acid groups offer also the advantage that sulfhydryl moieties can be easily attached to such polymers via the formation of amide bonds. Appropriate ligands are cysteine, homocysteine and cysteamine. The formation of amide bonds can be mediated by carbodiimides. Thiol oxidation during synthesis can be avoided as described above. The total amount of immobilized reduced and oxidized thiol groups can be determined in the same way as cationic thiomers.^[1]

Table1: Different type of thiomers and the effect on measured mucoadhesion^[2]

Polymer	Mucoadhesive bond strength
Chitosan–iminothioline	250-fold improved mucoadhesive properties
Poly(acrylic acid)–cysteine	100-fold improved mucoadhesive properties
Poly(acrylic acid)–homocysteine	Approximately 20-fold improved mucoadhesive properties
Chitosan–thioglycolic acid	Tenfold improved mucoadhesive properties
Chitosan–thioethylamidine	Ninefold improved mucoadhesive properties
Alginate–cysteine	Fourfold improved mucoadhesive properties
Poly(methacrylic acid)–cysteine	Improved cohesive and mucoadhesive properties
Sodium carboxymethylcellulose–cysteine	Improved mucoadhesive properties

Mucoadhesive properties of thiomers: Thiomers are capable of forming disulfide bonds with cysteine substructures by the formation of mucus gel layer covering mucosal membranes. Because of this property they exhibit higher mucoadhesive properties in comparison to the corresponding unthiolated polymers.

Thiomers are hydrophilic in nature they exhibit free thiol groups on the polymeric backbone. Due to these functional groups various properties of well-established polymeric excipients such as poly (acrylic acid) and chitosan are strongly improved^[3].

Molecular disulfide interaction: Modified alginate increases viscosity with time due to creation of intermolecular disulfide bonds. In order to quantify the visual observation rheology experiments were performed which indicate that the ratio between the shear storage modulus G' (elastic component) and the shear loss modulus G'' (viscous component) of alginate and alginate thiol solutions. Alginate thiol behaves as a gel, with storage modulus larger than the loss modulus. On the contrary, alginate shows the property of a viscoelastic solution. This result approved the existence of inter-molecular sulfide bond between alginate thiol polymer chains that may be potentially prevent further sulfide interactions with the mucus content^[4]

Enzyme inhibitory properties: Thiomers is the group of enzyme inhibiting polymers. They found a strong reduction of albumin degradation by a mixture of proteases in the presence of carbomer 934P. PCP and carbopol 934P were potent inhibitors of the proteolytic enzymes trypsin, α -chymotrypsin and carboxypeptidase A. As a result of the covalent attachment of cysteine to PCP, the inhibitory effect of the polymer towards carboxypeptidase A, carboxypeptidase B and chymotrypsin could be significantly improved. PCP Cys also had a significantly greater inhibitory effect than unmodified PCP on the activity of isolated aminopeptidase N present on intact intestinal mucosa.

The inhibitory effect of thiolated polymers also tested on intact vaginal mucosa and on buccal mucosa. Both thiolated and unmodified PCP significantly inhibited the hydrolysis of the synthetic substrate leu-*p*-nitroanilide by aminopeptidases present on the buccal mucosa. Thiolated PCP was thereby significantly more effective than the unmodified polymer^[5]

Compared to delivery systems based on the co-administration of enzyme inhibitors, thiomers offer the advantage that the inhibitory effect can be concentrated and localised on the delivery system. Hence, systemic toxic side effects as well as feedback regulations leading to an increased enzymatic activity can be avoided.

Peptide delivery systems based on thiomers^[6]

(a) In situ gelling formulations: The sol-gel conversion can be induced by differ in pH, in temperature or in electrolyte concentration. Thiolated polymers represent a new type of in situ gelling polymers. At physiological pH values, sufficiently high amounts of negative thiolate anions are present within the polymer representing the active form for oxidation. This oxidation leads to the formation of inter- and intramolecular disulfide bonds being responsible for an increase in viscosity. The in situ-gelling properties of deacetylated gellan gum, for instance, which shows a strong increase in viscosity in the presence of electrolytes could be significantly improved by the immobilisation of thiol groups. Furthermore, PAA-Cys, chitosan TGA and chitosan TBA have shown excellent in-situ gelling properties, with a clear correlation between the total amount of polymer-linked thiol groups and the increase in viscosity of the formed gel.

(b) Microparticles: Chitosan microparticles increase the stability by addition of multivalent anions, but as a result decrease mucoadhesion. The use of thiomers polymers like PAA₄₅₀-Cys for microparticle preparation increased cohesive properties. They increase stability by the formation of intramolecular disulfide bonds within the microparticles during the preparation process. After that a controlled drug release microparticles can be achieved. The release of the peptide drug can be increased by the addition of hydrophobic excipients like Eudragit RS[®] to the polymer. Disintegration studies increases stability of these thiomeric microparticles over 24 h, while particles comprising unmodified poly(acrylic acid) disintegrated within minutes.

Due to the immobilisation of thiol groups on microparticles the mucoadhesive properties are additionally improved. PAA₄₅₀-Cys microparticles, for instance, were almost 15-times more mucoadhesive on the intestinal mucosa than unmodified polymer particles.

(c) Liquid formulations: Thiomers mainly stable when stored in dry condition. In aqueous solutions, they form disulfide bonds in a pH-dependent. Due to instability in aqueous solutions thiomers not used in liquid formulations. Recently in new research thiomers to be stabilized in aqueous solutions but produced in inert conditions and the vessels are packed in an aluminium foil having oxygen scavenger such as iron-oxides. Based on this technology first mucoadhesive liquid formulations comprising thiomers were prepared and tested in vivo.

(d) Tablets: Tablets are approximately 40% of all dosage forms. Due to their convenient route of administration and their long-lasting shelf life patient compliance is very high. Depending on the drug carrier matrix and the auxiliary agents used, peptide liberation can be adjusted to delay or prolong release. If polymers are used as drug carrier matrices for tablets, the polymer forms a gel after contact with the liquids of mucosal membranes. In order to guarantee a swelling of orally given tablets on the intestinal mucosa, tablets can be enteric coated or in the case of stomach targeted delivery systems, coating with triglycerides was shown to be sufficient to provide a swelling of the dosage form once it reached the stomach. In addition, thiolated poly (methacrylic acid)/starch compositions were shown to swell only at pH>5 even without an enteric coating. The thickness of the gel layer controls on the one hand the diffusion of the peptide out of the polymer-matrix and hinders on the other hand the diffusion of peptidases into the swollen polymeric network. This swollen polymeric network is much less effective, if it disintegrates before the peptide diffuses out of it. Therefore only polymers with strong cohesive properties used as peptide drug carrier systems can guarantee a diffusion controlled release. In the gastrointestinal tract, however, mucoadhesion of polymer tablets is thought to be limited mainly because of peristalsis.

Stability ^[7]

Chemical stability of thiomers: Because of the sensitivity of thiol groups towards oxidation, the chemical stability of thiomers has already been investigated in detail. PCP-Cys and chitosan-TGA were tested both as representative anionic and cationic candidates, respectively. The polymers were tested in the form of freeze-dried powders and matrix-tablets. Polymers were stored for a period of 6 months at four different storage conditions, namely at -20°C (56% relative humidity; RH), at 4°C (53% RH), at 20°C (70% RH), and at 22°C (25% RH). Samples were taken after 6 months to determine the formation of disulfide bonds and the remaining thiol groups on the polymer. When the PCP Cys and chitosan TGA conjugate were stored in the form of a powder, a decrease in free thiol groups was observed only after storage at 20°C and 70% RH. Both polymers were found to be stable under all storage conditions when compressed into matrix-tablets.

Stability of peptides incorporated in to thiomers:

Another aspect of stability focuses on the stability of the therapeutic peptide being incorporated in a thiomeric carrier matrix. As most peptide drugs bear thiol and/or disulfide bonds in their chemical structure, thiol/disulfide exchange reactions with thiomers cannot be excluded a priori. Studies investigating such peptide-thiomer interactions, however, revealed that they take place only to a very limited extent. Moreover, for many therapeutic peptides such interactions can be excluded completely. Although generalisations must always be viewed with great caution, thiol/disulfide exchange reactions do not seem to take place if at least one of following demands is fulfilled:

- Solid delivery systems with no or comparatively low water content are generated
- The pH of the thiomers is below 5 leading to a marginal ratio of thiolate anions, which are the functional groups being responsible for thiol/disulfide interactions and oxidation processes
- The thiol/disulfide moieties of the therapeutic peptide being embedded in an anionic thiomers are neighbored by non-ionic or anionic amino acids
- The thiol/disulfide moieties of the therapeutic peptide being embedded in a cationic thiomers are neighbored by non-ionic or cationic amino acids

Evidence for these theories is not only provided by various *in vitro* studies but also by biofeedback studies in different animal species with different peptide drugs demonstrating that these therapeutic agents do not lose their efficacy having been embedded in a thiomers.

Thiolated quaternary ammonium chitosan conjugates for enhanced precorneal retention, transcorneal permeation and intraocular absorption of dexamethasone.

Application of Thiomers:

1. In Buccal Drug Delivery ^[8]: Due to the immobilization of thiol groups on poly(acrylic acid) the mucoadhesive properties of the corresponding microparticles were 3-fold improved. On the basis, due to the immobilization of thiol groups on well-established polymers their mucoadhesive properties are even further improved, although micro and nanoparticles being based on thiolated polymers do not disintegrate. Because of the formation of disulfide bonds within the polymeric network, the particles are stabilized consequently, also a controlled drug release out of thiomers micro and nanoparticles can be provided. Recently, microparticles comprising poly (acrylate) cysteine were generated via the solvent evaporation emulsification method. Particles were of spherical shape and partially porous structure and had a main size in the range of 20–60 Å with a center at 35 Å. Because of the formation of disulfide bonds within the particles they did not disintegrate under physiological conditions within 48 hr.

2. Permeation enhancing polymers in oral delivery of hydrophilic macromolecules: (thiomers/GSH systems) ^[9]: Thiolated polymers (thiomers) in combination with reduced glutathione (GSH) were shown to improve the uptake of hydrophilic macromolecules from the GI tract. The mechanism responsible for this permeation enhancing effect seems to be based on the thiol groups of the polymer. These groups inhibit protein tyrosine phosphatase, being involved in the closing process of tight junctions, via a GSH-mediated mechanism. The strong permeation enhancing effect of various thiomers/GSH systems such as poly(acrylic acid)–cysteine/GSH or chitosan-4-thio-butylamidine (chitosan–TBA)/GSH could be shown via permeation studies on freshly excised intestinal mucosa in Ussing type chambers.

3. Ophthalmic application ^[10]: An excellent biocompatibility and safe toxicological profile of chitosan-thiomers raw material was indicated in *in vitro* and *in vivo* studies. Efficacy studies in dry eye mouse models suggest that chitosan-thiomers eye drops may have some protective ocular surface properties. Additionally, it could be demonstrated that the residence time on the ocular surface of the new isotonic, buffered chitosan-thiomers eye drops in a rabbit model is increased in comparison with aqueous solutions and formulations containing unmodified chitosan.

4. Vaginal drug delivery systems ^[11]: The addition of clotrimazole had also an impact on the adhesion time of chitosan–TGA conjugate B, which remained 26-times longer on vaginal mucosa than the corresponding unmodified polymer. The immobilization of thiol groups guarantees a controlled drug release. Results of this study demonstrate that these new chitosan–

TGA conjugates are very promising vehicles for the vaginal application of clotrimazole in treatment of mycotic infections.

5. Synthesis and in vitro antitumor effect of diclofenac and fenoprofen thiolated and nonthiolated polyaspartamide-drug conjugates: ^[12]

Synthesis and antiproliferative effects of new thiomers–diclofenac and fenoprofen conjugates, hydrophilic, bioadhesive, polymeric prodrugs, as well as antiproliferative effects of diclofenac, fenoprofen and a series of previously described polymer–fenoprofen conjugates on five tumor cell lines. Thiolated and nonthiolated polyaspartamides were the chosen polymeric components. Drug-loading ranged from 5.6 to 22.4% , and the amount of SH groups ranged from 6.9 to 45.6 $\mu\text{mol g}^{-1}$. Tensile studies demonstrated a clear correlation between the amount of thiol and the mucoadhesive properties of the conjugates. The growth-inhibitory activity of the tested polymer–drug conjugates demonstrates that polyaspartamide-type polymers, especially thiolated polymers, enable inhibition of tumor cell growth with significantly lower doses of the active substance.

CONCLUSION

The immobilisation of thiol-bearing compounds on polymeric excipients such as poly (acrylates) and chitosans improve in their mucoadhesive property, enzyme inhibitory and permeation enhancing properties. As the cohesive properties are also strongly improved because of a cross-linking process by disulfide bond formation within the polymeric network, a mainly diffusion-controlled sustained release of thiomers embedded peptide drugs can be guaranteed. In comparison to non-invasive peptide delivery systems comprising unthiolated multifunctional polymers, the efficacy of delivery systems comprising the corresponding thiolated version is therefore significantly improved. According to these results, thiomers subscribe new generation of multifunctional polymers for non-invasive peptide delivery.

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