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Thiomers — From bench to market

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ABSTRACT

Thiolated polymers or designated thiomers are obtained by immobilization of sulhydryl bearing ligands on the polymeric backbone of well-established polymers such as poly(acrylates) or chitosans. This functionalization leads to significantly improved mucoadhesive properties compared to the corresponding unmodified polymers, as disulfide bonds between thiol groups of thiomers and cysteine-rich glycoproteins of the mucus gel layer are formed. Furthermore, enzyme- and efflux-pump inhibiting as well as improved permeation-enhancing properties are advantages of thiolization. By the covalent attachment of mercaptonicotinamide substructures *via* disulfide bonds to thiolated polymers these properties are even substantially further improved and stability towards oxidation even in aqueous media can be provided. Meanwhile, more than 50 research groups worldwide are working on thiolated polymers. For certain thiomers the scale up process for industrial production has already been done and GMP material is available. Furthermore, safety of thiolated poly(acrylic acid), thiolated chitosan and thiolated hyaluronic acid could be demonstrated *via* orientating studies in human volunteers and *via* various clinical trials. The first product (Lacrimera® eye drops, Croma-Pharma) containing a chitosan–N-acetylcysteine conjugate for treatment of dry eye syndrome will enter the European market this year. It is the only product providing a sustained protective effect on the ocular surface due to its comparatively much more prolonged residence time worldwide. Various further products utilizing, for instance, thiolated hyaluronic acid in ocular surgery are in the pipeline.

1. Introduction

In the late 1990s the concept of thiolated polymers – designated thiomers – was pioneered aiming to improve the mucoadhesive properties of well-established polymers [1]. In the following years thiomers turned out to be not just most mucoadhesive, but also exhibited various other features that are of therapeutic relevance and particular interest for the development of comparatively more efficient drug delivery systems. Apart from their mucoadhesive properties, *in situ* gelling, permeation enhancing and efflux pump inhibiting properties are nowadays the focus of academic and industrial research worldwide. Evidence for their potential is provided by almost 500 articles of around 50 different research groups demonstrating their superiority over the corresponding unmodified polymers. Furthermore, the first commercial product containing a thiomer (Lacrimera®; Croma-Pharma) will reach the European and Canadian market this year. This review shall provide an overview over the different types and functions of thiolated polymers inspiring further research groups to utilize this promising technology.

2. Synthesis of thiomers

Generally, reactivity of thiomers is strongly dependent on the pKa value of the thiol group of the chosen ligand. The lower the pKa value the more thiolate anions are available at physiological pH representing the reactive form of sulfhydryls. The following rank order of increasing reactivity might be helpful in this connection: mercaptobenzoic acid (pKa = 6.2) > 4-aminothiophenol (pKa = 6.86) > mercaptophenylacetic acid (pKa = 7.7) > N-acetylcysteine (pKa = 8.2) > cysteamine (pKa = 8.3) > cysteine (pKa = 8.4) > glutathione (pKa = 8.8) > homocysteine (pKa = 10.0) > thioglycolic acid (pKa = 10.3). The more reactive thiol groups are the more rapidly and to a higher extent disulfide bonds are formed within the thiomers and with cysteine-substructures of biological materials. According to this, reactivity of thiol groups has a great impact on synthesis, storage stability and *in vivo* performance. For the synthesis of thiomers the covalent attachment of thiol bearing ligands to polymeric backbones *via* amide and amidine bond formation is the most common method. More recently, other synthetic approaches were established to generate thiomers. Resulting thiolated polymers are purified *via* dialysis or repeated precipitation followed by lyophilization or evaporation. For certain thiomers, such as thiolated chitosan and thiolated hyaluronic acid the scale up for a commercial production has already been done and GMP material is available.

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2.1. Amide bond formation

Thiolation *via* amide bond formation is mostly mediated by carbodiimides and can be performed with polymers exhibiting either carboxylic acid groups or primary amino groups (Fig. 1). The thiol bearing molecules cysteine [2] and cyteamine [3] are likely most often used for thiolation of carboxylic acid groups bearing polymers such as poly(acrylic) acid, pectin or alginates [4–6]. In case of primary amino groups bearing polymers in particular chitosan can be modified with ligands such as thioglycolic acid or N-acetylcysteine [7]. Here, an activated o-acylurea is formed between the carbodiimide and the carboxylic acid group of the ligand leading to the formation of amide bonds with the amino groups of the glucosamine subunits in this polymer [8]. Utilizing cysteine as ligand bears the problem of unintended side reactions, as the formation of polymer-cysteine-cysteine$_n$ side chains cannot be avoided. For industrial thiomer production, N- or C-protected cysteine, such as N-acetyl cysteine being utilized for the synthesis of thiolated chitosan, is used. By these methods, generally coupling rates in the range of 50–500 μmol thiol groups per gram of polymer can be achieved.

2.2. Amidine bond formation

Establishing amidine bonds between an amino group of polymers, such as chitosan, polyethylene imines [9] or dendrimers [10] and thiolated imidates, namely isopropyl-S-acetylthioacetimidate or iminothiolane (Traut's reagent), is another way to synthesize thiolated polymers [11–14] (Fig. 2). As 4-thiobutylamidine substructures turned out to be chemically unstable, isopropyl-S-acetylthioacetimidate is preferred over iminothiolane as coupling reagent [15]. The mechanism behind this reaction is a nucleophilic reaction between primary amines or amino groups and the imidates resulting in the mentioned amidine bonds [13]. As amidine substructures have a more pronounced cationic character than amines, the overall cationic character of polymers is thereby raised. In case of chitosans, for instance, this effect is advantageous, as the unmodified polymer precipitates at a pH > 6, whereas thiolated chitosans exhibiting amidine substructures do so at a pH > 6.5–7.5 depending on the degree of modification [11]. Utilizing this technique approximately 250 μmol thiol groups per gram of polymer can be immobilized.

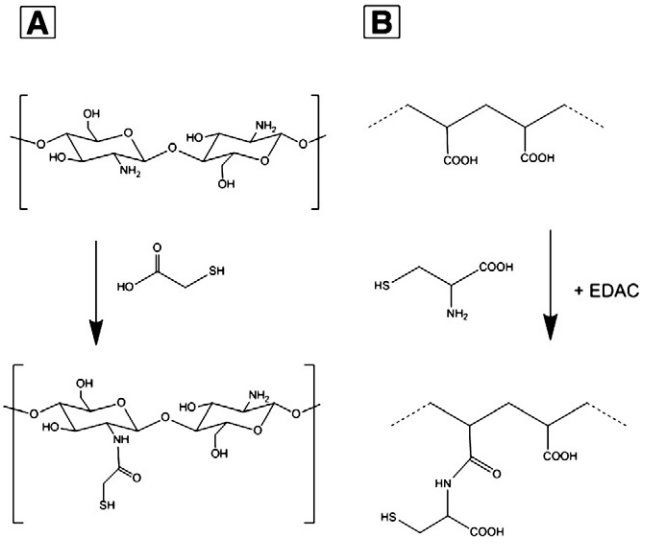

Fig. 1. Syntheses of thiomers *via* amide bond formation: The illustrated syntheses are based (A) on the cationic polymer chitosan and (B) on the anionic polymer poly(acrylic acid).

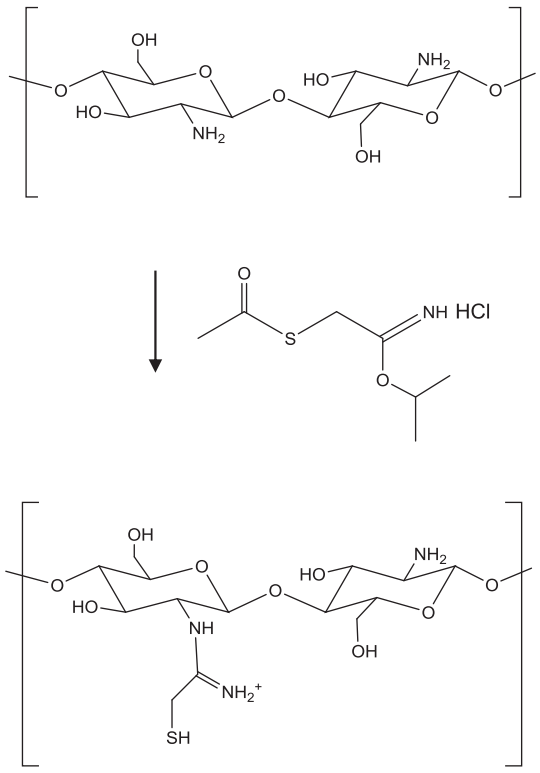

Fig. 2. Synthesis of thiomers *via* amidine bond formation.

2.3. Amine bond formation

The periodate cleavage of vicinal diols followed by reductive amination is a useful technique for thiolation of polysaccharides that do not exhibit carboxylic acid groups or primary amino groups. Due to the introduction of amino groups, non-ionic polymers are transformed to cationic polymers. Hydroxyl groups on neighboring carbon atoms, so called vicinal diols, are present in many polysaccharides, such as cellulose ethers. Even though these polysaccharides display just hydroxyl groups, they are accessible for thiolation. In a two-step synthesis, the vicinal diol moiety is first cleaved with sodium periodate resulting in the correspondent dialdehyde. Treatment with thiol bearing amines, such as cysteamine, under reductive conditions results in a secondary amine bond between the thiol bearing ligand and the polymer [16,17]. Periodate cleavage is highly selective to the neighboring hydroxyl groups, and excess reagent can be easily inactivated with ethylene glycol. As ethylene glycol is deemed toxic for biopharmaceutical applications, however, it will certainly have to be substituted in case of an upscale process. Thiolation of hydroxyethylcellulose, for instance, was feasible following this procedure resulting in a cationic thiomer [17] (Fig. 3). Coupling rates using this synthetic approach are in the range of 100–1500 μmol thiol groups per gram of polymer.

2.4. Conversion of hydroxyl groups into thiol groups

Polymers lacking the above-mentioned functionalities can nonetheless render thiomers by direct conversion of polymeric hydroxylic moieties. This is even more important in cases where the ionic character of thiomers is facing problems [18], such as ionic interactions with oppositely charged drugs, unintended pH dependent drug release or poor crosslinking due to ionic repulsion. Therefore, non-ionic thiomers are also of interest. In order to gain such thiomers, hydroxyl groups of polymers are first substituted by bromine, which is in the presence

of thiourea transformed into thiol groups. So far, primary and secondary hydroxylic groups in hydroxyethylcellulose (HEC) were successfully replaced by sulfhydryl groups resulting in thiolated HEC. This procedure requires bromination of this cellulose derivative mediated by N-bromosuccinimide-triphenylphosphine in lithium bromide *N*,*N*-dimethylacetamide (DMA) leading to the intermediate bromo-hydroxyethylcellulose [19]. Nucleophilic substitution of hydroxylic groups with bromine, which is believed to take place through the formation of alkoxyphosphonium salts followed by attack of the ylide ions on to the phosphonium ester bonds, is considered the mechanistic background of this reaction [20]. In a second step, the thiol group is introduced by treating the intermediate bromo-hydroxyethylcellulose with thiourea [19]. Fig. 3 depicts the synthetic pathway to thiolated hydroxyethylcellulose. Regarding the degree of modification, approximately 130 μmol thiol groups can be coupled per gram of polymer.

2.5. Preactivated thiomers

Susceptibility towards oxidation is the main problem of thiolated polymers. Generally, thiomers are stable in dry form such as cysteine and most other thiols. In aqueous solution, however, formation of inter- and intramolecular disulfide bonds occurs particularly in the presence of oxidants, such as oxygen at pH > 5. To protect thiol groups from uncontrolled oxidation reactions, the concept of S-protected thiomers was established by our research group. Adapted from covalent chromatography, where proteins or peptides are covalently linked to thiol bearing resins, such as thiopropylsepharose, thiol groups in thiomers were activated using mercaptopyridines. Formation of mixed disulfides with pyridyl substructures can be easily achieved over a broad pH range and the mercaptopyridine moiety is easily released resulting in free thiol groups [21]. Hence, the concept of S-protection also provides full reactivity of the thiol groups. To emphasize the preserved and improved reactivity, S-protected thiomers are also designated as preactivated thiomers. For safety reasons, mercaptonicotinamide and mercaptonicotinic acid are preferred over mercaptopyridine as ligand [5,22].

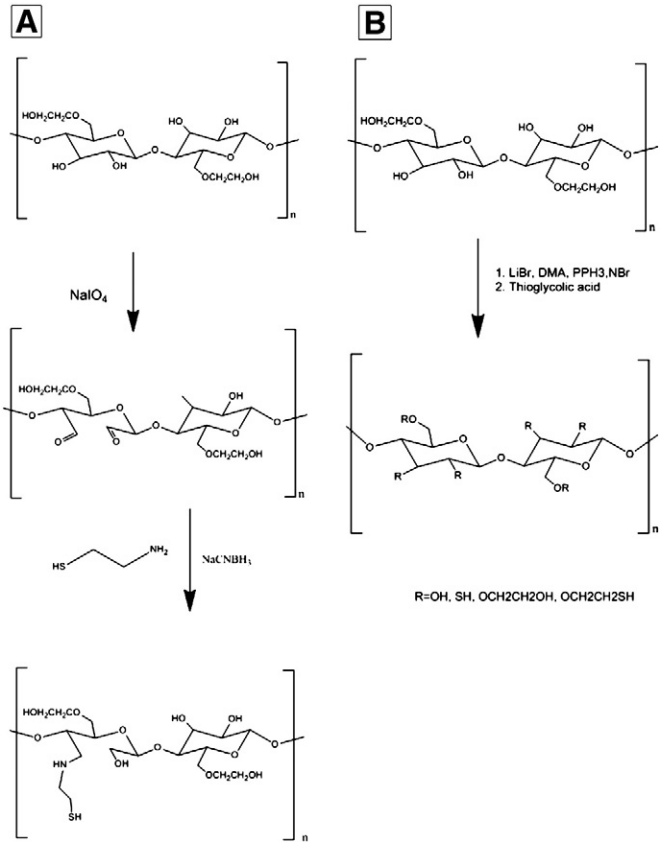

Fig. 3. Novel synthetic approaches to thiomers based on hydroxyethylcellulose; A: amine bond formation and B: conversion of hydroxyl groups into a sulfhydryl groups.

In general, preactivated thiomers are obtained in a two-step reaction. First, the thiomer is generated *via* one of the synthetic pathways described above. Then, preactivation is achieved by formation of disulfide bonds using ligands with a mercaptopyridine substructure. Preactivated thiolated poly(acrylic acid) for example was synthesized by coupling L-cysteine to the polymeric backbone followed by preactivation with 2-mercaptonicotinic acid [5].

Recently Hintzen et al. established another synthesis to gain entirely S-protected thiomers. Entirely S-protected pectin was synthesized by immobilization of adducts between L-cysteine and 2-mercaptonicotinic acid, which were obtained *via* formation of mixed disulfide bonds between the two compounds in a precedent step [6].

2.6. Polymerization of S-protected thiolated monomers

Preactivated thiomers obtained *via* polymerization of S-protected thiolated monomers was described by Sohli et al. In a step prior to the polymerization reaction, the intermediate 6-(2-amino ethyldisulfanyl) nicotinic acid was prepared *via* disulfide bond formation between 6-mercaptonicotinic acid and cysteamine. Then, this intermediate was converted to 6-(2-acryloylamino-ethyldisulfanyl)-nicotinic acid according to the Schotten–Baumann reaction followed by copolymerization with acrylic acid [23] (Fig. 4). By this method, coupling rates in the range of 400–500 μmol per gram of polymer can be achieved. As the S-protected thiolated monomer can be co-polymerized with numerous other monomers, this method opens the door to a huge variety of further thiomers.

2.7. Chemical characterization of thiomers and preactivated thiomers

The total amount of immobilized thiol groups including also already oxidized thiol groups can be quantified photometrically with Ellman's reagent (5,5′-dithio-bis(2-nitrobenzoic acid)) after reduction with sodium borohydrate [1]. Without the reduction process, the amount of free thiol groups can be determined. In case of coupling reactions where amide bonds are formed with amino-ligands such as cysteine or cysteamine, the remaining amount of non-conjugated ligand in the polymer can be quantified with 2,4,6-trinitrobenzenesulfonic acid (TNBS) [24]. Assessment of preactivated thiol groups occurs spectrophotometrically. By addition of glutathione, the aromatic thiol bearing ligand is released from the polymer and the resulting shift in absorbance is quantified [5]. Furthermore, evidence for thiolation can be provided for certain thiomers *via* ^{1}H-NMR and FTIR-analyses [23].

3. Functions of thiomers and S-protected thiomers

3.1. Mucoadhesive properties

Generally, mucoadhesion is provided by non-covalent bonds, such as hydrogen bonds, van der Waal's forces and ionic interactions or simply due to physical interpenetration effects [25]. In contrast to such rather weak bonds, thiomers and S-protected thiomers offer the advantage of covalent bonds with cysteine-rich subdomains in the mucus gel layer *via* formation of disulfide bonds due to thiol/disulfide exchange reactions and oxidation processes [26]. Furthermore, thiomers exhibit crosslinking properties *via* disulfide bond formations taking place in a time dependent and controllable manner within their polymeric network. Due to this process, on the one hand, sufficiently high cohesive properties within the polymer are provided avoiding an adhesive bond failure within the thiomer. On the other hand, thiomers can interpenetrate the mucus gel layer comparatively more efficiently

forming preferably after this process stabilizing and anchoring disulfide bonds within the mucus network. Thiomers exhibit therefore much stronger mucoadhesive properties as the corresponding unmodified polymers [8,27,28], having meanwhile been shown by numerous research groups worldwide [29–32].

These findings could be further confirmed by an *in vivo* experiment, where Barthelmes et al., for instance, evaluated the mucoadhesive properties of thiolated nanoparticles. Nanoparticles composed of chitosan–thioglycolic acid (chitosan–TGA) loaded with fluorescein diacetate (FDA) were applied to the urinary bladder of rats. After an incubation of 6 h, the percentage of remaining particles on the intravesical mucosa was determined. Thereby, thiolated chitosan nanoparticles showed a 4-fold higher mucoadhesion compared to unmodified chitosan nanoparticles (Fig. 5) [33].

In another *in vivo* study, the ocular residence time and biodistribution of eye drops containing thiolated chitosan was evaluated in rabbits. Distribution of the ^{124}I-labeled chitosan–N-acetylcystein (chitosan–NAC) was thereby assessed using microPET technology. In comparison to an aqueous solution of Na^{124}I, which was rapidly removed, ^{124}I-labeled chitosan–NAC remained on the ocular surface during the whole experiment. MicroPET images of this experiment are depicted in Fig. 6 [34].

In addition, mucoadhesive properties provide the possibility to use thiomers as antiperspirants substituting aluminum salts, which are meanwhile under suspicion of causing Alzheimer's disease and breast cancer. In a European patent, thiolated polyethylene imine, incorporated in different carrier substances, is stated to act as a pore blocker in the eccrine sweat glands after swelling therein. Mucoadhesion is believed to be at least one trigger for the antiperspirant activity [35].

Even further improvement regarding mucoadhesive properties could be achieved by preactivated thiomers [6,36,37]. Full reactivity of thiol groups towards cysteine residues in mucus layers is preserved with this strategy, as the presence of disulfide bonds prevent premature oxidation within the polymers. Furthermore, preactivated thiomers react in a more sufficient way with thiol groups in the mucus than the thiol/disulfide exchange reactions taking place in the case of conventional thiomers. Iqbal et al. studied the *in vitro* mucoadhesive properties of poly(acrylic acid), thiolated poly(acrylic acid) and the corresponding preactivated thiomer. Tensile studies as well as the rotating cylinder method – two entirely different experiments – showed correlating outcomes regarding the tested polymers. The strongest adhesive features could be determined for the S-protected polymer, followed by the thiomer and the unmodified polymer. These results were further confirmed by an increase in viscosity of polymer/mucin mixtures [5]. Another advantageous feature of preactivated thiomers is their pH-independent reactivity. The reactive form of thiomers is the thiolate anion. In the case of alkyl thiols, this form is mainly present at pH values slightly above physiological conditions, due to a pKa value of 8–10. Hence, thiomers could not be applied for gastric or vaginal mucoadhesive drug delivery systems, as their thiol groups are not sufficiently active at low pH values. Overcoming this shortcoming was possible with the strategy of preactivated thiomers. Recently, Hauptstein et al. demonstrated the applicability of S-protected thiolated pectin as mucoadhesive drug delivery system in the stomach [38].

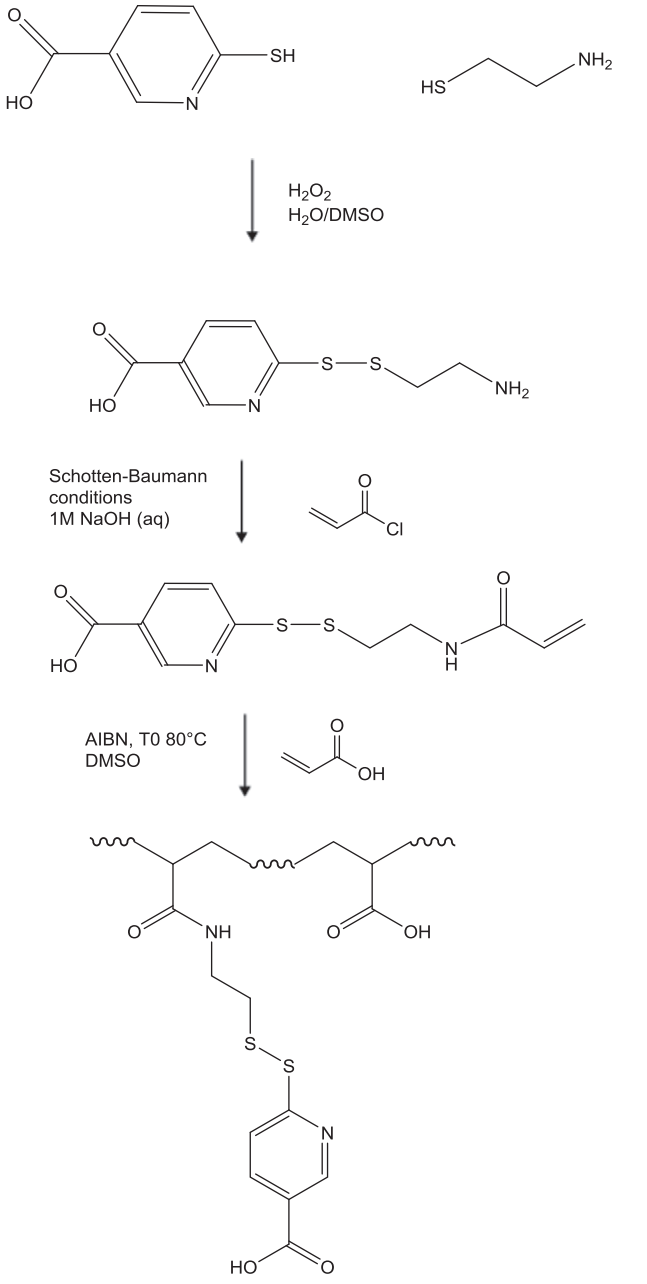

Fig. 4. Synthesis of S- protected thiomers *via* polymerization of S-protected thiolated monomers.

3.2. In situ gelation

The capability of *in situ* gel formation is especially of interest for liquid or semisolid vaginal, nasal and ocular formulations. Being easily applied in low viscous form, these formulations can provide a prolonged residence time after gelation on the site of application. Inter- and intramolecular disulfide bonds, which are formed due to oxidation processes at physiological pH values are responsible for the *in situ* gelling properties of thiolated polymers [39]. Sol–gel transition *in vitro* was evaluated by rheological measurements. Herein, a clear correlation between the total amount of coupled thiol groups and the increase in viscosity was observed, as the elastic modulus in thiomer solutions increased with increasing amounts of immobilized thiol groups [11,40]. Furthermore, the effect of various oxidizing agents on rheological properties of thiolated chitosan was evaluated by Sakloetsakun et al. An over 16,000-fold increase in dynamic viscosity was observed for chitosan–thioglycolic acid (CS–TGA), whereas the corresponding unmodified polymer did not show any changes in viscosity [41]. In accordance, Gyarmati et al. reported the successful synthesis of a redox- and pH responsive thiolated poly(aspartic acid) with reversible sol–gel transition [42]. In contrast to conventional thiomers, fully S-protected thiomers are stable towards oxidation. Even addition of hydrogen peroxide, for instance, did not influence rheological properties of solutions of entirely S-protected pectin and no significant differences in viscosity were observed after a storage period of six months [6].

3.3. Permeation enhancing properties

In numerous studies thiomers were shown to exhibit a permeation enhancing effect [43–46]. In comparison to medium chain fatty acids that can still be regarded as a kind of gold standard for orally used permeation enhancers, thiolated polycarbophil, for instance, was shown to exhibit a more pronounced effect when applied in the same concentration [47]. Results of this study are illustrated in Fig. 7.

Supposedly, the mechanism behind this permeation enhancing effect is the inhibition of protein tyrosine phosphatase (PTP), which is responsible for the dephosphorylation of tyrosine subunits in occludin. Occludin is an integral membrane protein of the tight junctions and dephosphorylation of this protein results in closure of tight junctions [48]. Consequently, inhibition of PTP results in a more phosphorylated occludin and therefore in an opening of tight junctions. The apical side of mucosal membranes displays glutathione in reduced (GSH) form as well as in oxidized (GSSG) form, of which GSH functions as inhibitor of PTP [49]. Thiolated polymers cause a shift in the GSH/GSSG balance in direction of GSH resulting in a reversible tight junction opening [50]. Glutathione as such, however, does not show a permeation enhancing effect, as it is likely too rapidly oxidized on the mucosa. Nevertheless, it could be shown in various studies that the addition of reduced glutathione to thiomers improves their permeation enhancing effect [47,51–59]. Iqbal et al., for instance, demonstrated within an *in vivo* study in rats, that chitosan–thioglycolic acid in combination with GSH could improve the absorption of orally administered leuprolide. In comparison to leuprolide in solution, a 3.72-fold increased area under the plasma concentration curve was obtained with the formulation containing the combination of thiomer and GSH [60]. Furthermore, this permeation enhancing effect of thiomers is even much higher when they are preactivated [22,61].

3.4. Efflux pump inhibition

Efflux pumps, such as P-glycoprotein (P-gp) and multidrug-resistance protein (MRP), in the apical membrane of enterocytes are a limiting factor for the oral bioavailability of many drugs. As these transporters function as protective shields against xenobiotics, a broad range of compounds such as anticancer drugs, antibiotics, calcium channel blockers or immunosuppressives are actively transported by efflux pumps from the inner side of the membrane to the outer side [62,63]. Inhibition of those transport proteins is therefore of great interest and can be achieved with polymers, such as polyethyleneglycols [64] or pluronic block copolymers [65]. Recently, P-gp inhibitory effects have also been demonstrated for various thiomers both *in vitro* and *in vivo* [22,45,66–69]. A comparative study about different polymeric and low molecular weight P-gp inhibitors using rhodamine 123 (Rho-123) as P-gp substrate, revealed a 3-fold higher uptake of rhodamine 123 for chitosan–4-thiobutylamidine in combination with GSH compared to a 1.8-fold improvement for Myrj 52. The advantage of thiomers over other polymeric efflux pump inhibitors, could also be confirmed by an *in vivo* experiment, where the bioavailability of Rho-123 was evaluated in rats (Fig. 8) [67].

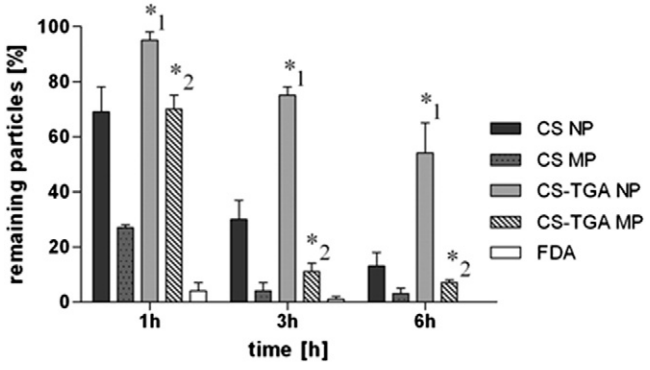

Fig. 5. Histogram shows the *in vivo* remaining percentage of fluorescein diacetate loaded particles composed of thiolated chitosan and unmodified chitosan on the intravesical mucosa of rats.
Adapted from: Barthelmes et al. [33].

Regarding the mechanism of action, thiomers are supposed to react specifically with cysteine subunits in one of the transmembrane regions of P-gp. Due to formation of covalent bonds, namely disulfide bonds, the transport mechanism might be blocked by thiomers [70].

In the case of preactivated thiomers, protection with thiolated pyridyl substructures leads to a more reactive form of thiol groups compared to alkyl thiols. At physiological conditions, alkyl thiols are only to a lower extent present in their reactive form, the thiolate anion, which is due to a pk_a value of 8–10. As a consequence, full reactivity cannot be reached at physiological pH values. In contrast, S-protected thiomers, like preactivated thiolated chitosan, display a pH independent reactivity and can therefore interact more efficiently with cysteine subunits in the transmembrane domains under formation of mixed disulfides. *In vitro* transport studies with thiolated and S-protected chitosan support this assumption, as increased Rho-123 permeation across a Caco-2 monolayer was observed for the preactivated thiomer [22].

4. Dosage forms based on thiomers and S-protected thiomers

4.1. Matrix tablets

Mucoadhesive matrix tablets offer potential in particular for intraoral, oral and vaginal drug administration [37,71–73]. *In situ* crosslinking properties of thiomers due to inter- and intramolecular disulfide bond formation provide greater cohesiveness and stability of the swollen polymer matrix [74]. Disintegration studies performed on tablets of poly(acrylic acid) in its unmodified and thiolated form revealed an overall stability for the thiomer. No disintegration could be observed for tablets compressed of poly(acrylic acid)-cysteine after 48 h, whereas tablets of the corresponding unmodified polymer disintegrated within 2 h [75]. Because of this comparatively much higher stability, a more controlled drug release can be achieved. In several *in vivo* studies, matrix tablets of thiomers and preactivated thiomers were evaluated as potential drug delivery systems. Oral administration of the GnRH antagonist antide in matrix tablets composed of chitosan–4-thiobutylamidine (chitosan–TBA) in combination with GSH resulted in a significant uptake of the peptide, compared to antide in solution. With a relative bioavailability of 3.2% in pigs, the efficacy of thiolated chitosan tablets could be demonstrated. In contrast, antide was not detectable in the plasma at all, when administered just in aqueous solution [76]. In a follow up study, oral bioavailability of antide tablets comprising thiolated as well as S-protected thiolated chitosan was evaluated. A relative bioavailability of 10.9%, a delayed maximum plasma concentration and a 421-fold increase in the area under the plasma concentration curves of antide was observed for matrix tablets of chitosan–thioglycolic acid-6-mercaptonicotinic acid compared to the peptide in solution [77].

Another study in humans evaluated the controlled release properties of matrix tablets based on thiomers. Fluorescein release from ocular inserts based on thiolated poly(acrylic acid)-cysteine or unmodified poly(acrylic acid) was studied in comparison to eye drops. Tolerability of the ocular insert was examined as well. Determining the mean fluorescein concentration in the cornea/tear film compartment revealed a fluorescein concentration on the ocular surface for more than 8 h in the case of the inserts based on thiolated PAA. In contrast, a rapid decline in the fluorescein concentration was visible for eye drops as well as inserts based on unmodified polymer [78]. Fig. 9 shows the inserts in the human lower cul de sac.

With regard to drug targeting, the concept of S-protected thiomers provided the possibility to generate gastroretentive mucoadhesive

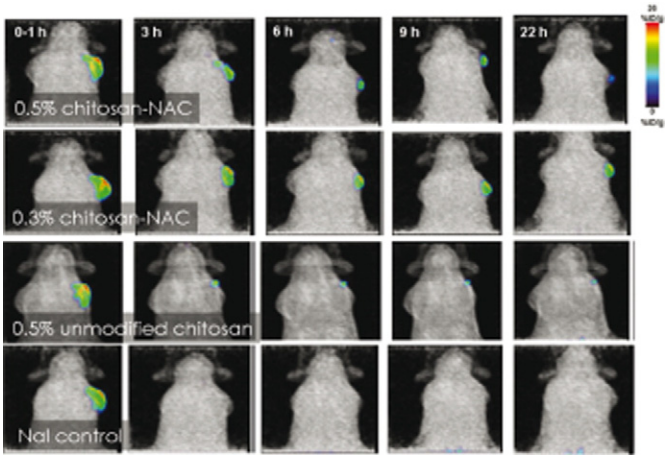

Fig. 6. MicroPET images of rabbits after topical application of eye drops containing ^{124}I labeled chitosan–NAC, unmodified chitosan and aqueous solution of $Na^{124}I$ as control. Adapted from: Dangl et al. [34].

matrix tablets for rosuvastatin calcium [38]. Gastroretention can be prolonged by mucoadhesive systems [79], such as preactivated thiolated pectin, as the S-protection enables formation of disulfide bonds with the gastric mucosa at low pH values. Compared to unmodified pectin, pectin-cystein-2-mercaptonicotinic acid showed 5-fold improved mucoadhesive properties respectively as determined by the enhancement ratio for the total work of adhesion (TWA) within tensile studies on porcine stomach at pH 1. However, only a 2-fold higher TWA was measured in the case of the corresponding thiomer, pectin-cysteine [80]. In contrast to S-protected thiomers, conventional thiomers display a reduced reactivity against thiol bearing mucus components and the concentration of thiol anion $-S^-$, being required as reactive form, is very low at pH values below 5 for thiomers of the first generation [81].

4.2. Micro- and nanoparticles

In comparison to single unit dosage forms, like tablets, multiple unit dosage forms such as micro- and nanoparticles are known to have *per se* a prolonged residence time in the small intestine. Coupe et al. demonstrated in human volunteers that after 3 h tablets have left the small intestine entirely, whereas more than 50% of particles were still remaining in the small intestine [82]. According to this, the mucoadhesive properties of thiomers should be further enhanced when being formulated to micro-/nanoparticles. Albrecht et al. demonstrated by *in vivo* magnetic resonance imaging the systemic uptake of the contrast agent diethylenepentaacetic acid gadolinium(III)dihydrogen salt (Gd-DTPA) from thiolated nanoparticles. After oral administration as nanoparticulate suspension, an increased MRI signal could be detected in the urinary bladder, indicating the renal elimination of Gd-DTPA after systemic uptake. In contrast, Gd-DTPA alone or with the unformulated thiomer did not cause an increase in the MRI signal [83]. Improved mucoadhesive properties on intestinal mucosa could be demonstrated for thiolated nanoparticles in comparison to unmodified nanoparticles. Studying the residence time on intravesical mucosa, thiolated micro- and nanoparticles displayed an increased mucoadhesion compared to their unmodified nanoparticles, as described in more detail in Section 3.1 [33].

In addition to improved mucoadhesion, nanoparticles composed of thiolated polymers can be covalently cross-linked after their preparation and display therefore a higher stability over particles stabilized *via* ionic interactions. Chitosan nanoparticles, for instance, are prepared by *in situ* gelation of ionic polymers with a negatively charged polyanion, such as tripolyphosphate (TPP). This ionic crosslinking, however, can lead to rapid disintegration at low pH values and therefore to a rapid release of encapsulated drugs. In contrast, chitosan–TGA nanoparticles, obtained by ionic gelation with TPP, can be oxidized with hydrogen peroxide leading to the formation of disulfide bonds. This covalent crosslinking allows the removal of the ionic cross-linker TPP *via* dialysis. Concerning stability in body fluids, 99% of covalently cross-linked chitosan–TGA nanoparticles were found to be stable in simulated gastric fluid up to 60 min, whereas 90% of unmodified and not oxidized particles disintegrated within the first ten minutes [84]. Despite their stability in body fluids, disintegration of these covalently cross-linked nanoparticles can occur in reductive environment, as for example in the presence of GSH in the cytoplasm, which leads to a cleavage of the disulfide bonds. Therefore, this reversible cross-linking is suitable for targeted delivery of, for instance, plasmid DNA to the cytoplasm [85]. In studies on transfection efficiency of chitosan–TGA/DNA nanocomplexes, Lee et al. observed that thiolated chitosan nanoparticles induced significantly higher gene expression compared to unmodified chitosan. Especially cross-linked chitosan–TGA/DNA nanoparticles displayed a sustained DNA release and continuous expression up to 60 h after transfection. In an *in vivo* study on mice, significantly higher gene expression in BAL (broncho alveolar lavage) cells was observed 14 days after intranasal administration of chitosan–TGA/DNA nanoparticles, compared to non cross-linked nanoparticles and unmodified particles. Results are depicted in Fig. 10 [86].

In another study, thiolated poly(amido ethylenediamine) mediated gene delivery improved the efficacy of ischemia-inducible VEGF gene therapy in rabbits [87].

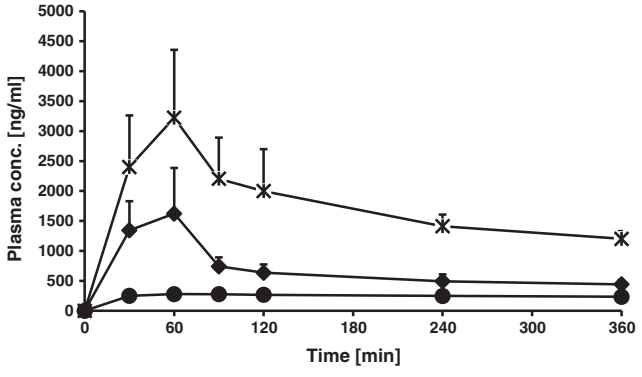

Fig. 7. Plasma concentration of FD4 after oral administration in aqueous solution (•) and as tablets with A sodium caprate (♦) and B polycarbophil-cysteine/GSH (x). Adapted from: Perera et al. [47].

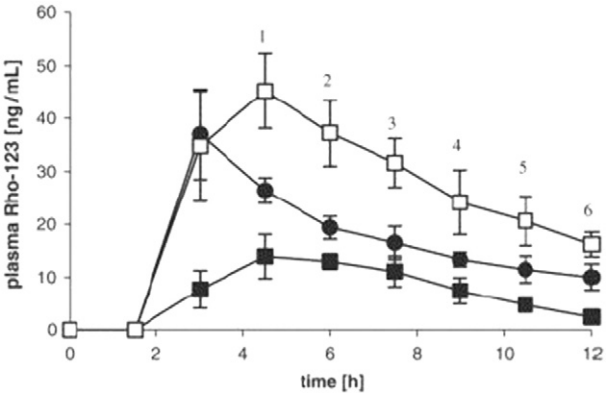

Fig. 8. Illustration of plasma concentration after oral administration of the P-gp substrate rhodamine123 in matrix tablets of thiolated chitosan (□), PluronicP85 (■) and Myrj 52. Adapted from: Föger et al. [67].

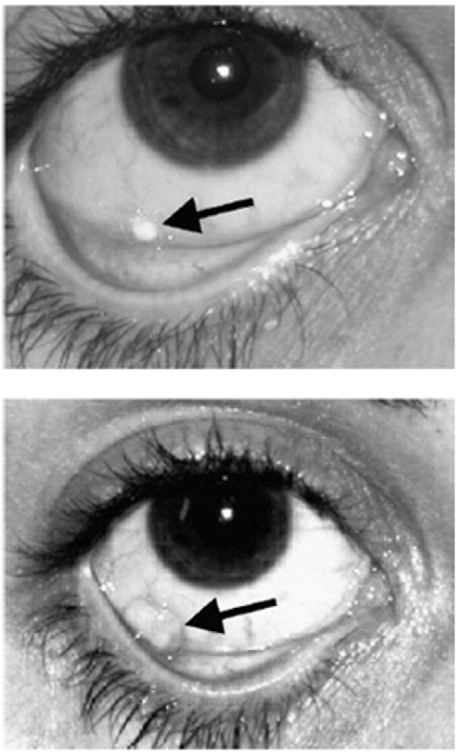

Fig. 9. PAA450–Cys insert in the lower cul de sac; immediately after application (upper picture) and 8 h after application (lower picture).
Adapted from: Hornof et al. [78].

4.3. Viscous liquids and gels

Reduced stability in aqueous solutions due to disulfide bond formation is a major drawback of thiomers, although, production under inert conditions as well as packing to the exclusion of light and oxygen allowed the preparation of stable liquid thiomer formulations. In contrast, entirely S-protected thiomers were shown to be stable in aqueous solutions [6]. Regarding drug delivery systems, liquid formulations are in general frequently used in eye drops or nasal sprays, as low viscous formulations are more comfortable for the patients to apply. Hydrogels, on the other hand, can provide a prolonged residence time and on the site of application due to their higher viscosity. Based on their *in situ* gelation and mucoadhesive properties thiomers are promising excipients as drug delivery systems in the form of hydrogels for intraoral, nasal, ocular and vaginal delivery. Recently, Friedl et al. demonstrated the suitability of a hydrogel formulation composed of partially S-protected thiomer as vaginal delivery system for nystatin. Within this study, gels based on thiolated and S-protected thiolated chitosan were compared with commercially available formulations over 24 h in a test system simulating vaginal conditions. With a 1.5-fold improved mucoadhesion – determined by the residence time on the vaginal mucosa – the preactivated thiomer performed superiorly over the commercially available formulations. This is advantageous, as an extended vaginal residence time can reduce dosing frequency and consequently ensure higher patient compliance [88].

4.4. Novel dosage forms containing thiomers

Besides the formulations described above, new dosage forms for thiomers and preactivated thiomers have been developed. A successful coating of liposomes with thiolated poly(acrylic acid) was first described by Werle et al. [89]. Later on, Gradauer et al. developed liposomes coated with thiolated and S-protected thiolated chitosan, whereby coupling occurred *via* formation of a covalent bond between functionalized maleimide groups of liposomes and free thiol groups of the polymer [90]. These liposomes turned out to be a potent delivery system for the oral administration of salmon calcitonin. Drug loaded liposomes were either coated with chitosan–TGA or an S-protected version of the same polymer (CS–TGA-MNA) and bioavailability studies were performed after oral application to rats. Liposomes coated with CS–TGA-MNA caused the highest reduction in blood calcium level to 65% of the initial value after 6 h. In comparison to calcitonin in solution, an 8.2-fold increase in the area above the curve was obtained. The effect on the blood calcium level caused by the different formulations containing calcitonin is depicted in Fig. 11 [91].

Another strategy for drug delivery is provided by self nanoemulsifying drug delivery systems (SNEDDS), which are isotropic mixtures of oils, surfactants and co-surfactants. Administered as preconcentrates in capsules or as solution, they are rapidly dispersed to form droplets of approximately in the nano-range, when they are in contact with body fluids, such as the intestinal fluid [92,93]. Combining the strategy of thiomers and SNEDDS, an innovative drug delivery system for insulin could be generated. Chitosan–TGA was incorporated into a mixture of 65% (w/w) miglyol 840, 25% (w/w) cremophor EL and 10% (w/w) co-solvents (a mixture of DMSO and glycerol). This SNEDDS formulation was capable of protecting insulin against enzymatic degradation [94]. Latest developments include patches composed of thiolated chitosan [95], thiolated chitosan as scaffold in tissue engineering [96] as well as a bilayered ocular drug delivery system for gatifloxacin based on thiolated sodium alginate [97].

5. Clinical trials

After multiple *in vitro* experiments and *in vivo* studies in diverse animal models, thiomers have been evaluated in humans. In the field of ocular delivery, safety of thiolated hyaluronic acid in ocular implant is evaluated in ongoing clinical trials. Thereby, hyaluronan thiomer is implanted during combined cataract and non-penetrating deep sclerectomy surgery and this study is aimed to measure safety and efficacy up to 12 months. Efficacy analysis includes the postoperative reduction of the intra ocular pressure. Furthermore, the percentage of patients, who need additional medication to lower the intraocular pressure or additional surgical intervention are recorded [98]. Especially in the therapy of the dry eye syndrome, liquid thiomer formulations have shown great potential. Ocular mucins are a key factor in preserving hydration of the cornea and conjunctiva and a defective mucus layer is the main promoter in the development of a dry eye syndrome [99]. Tear substituents, which are mainly based on hydrophilic mucoadhesive polymers, such as carbomer

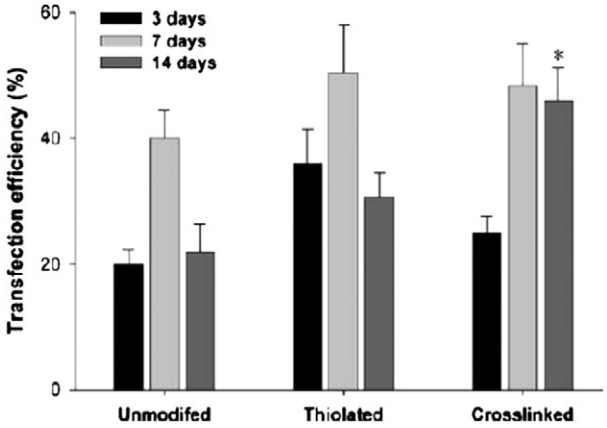

Fig. 10. Histogram illustrates the level of gene expression in BAL cells. Gene expression level was calculated by counting the number of total cells and green fluorescent protein expressing cells. *$P < 0.01$ relative to unmodified and thiolated chitosan at 14 days post-intranasal administration ($n = 4$).
Adapted from: Lee et al. [86].

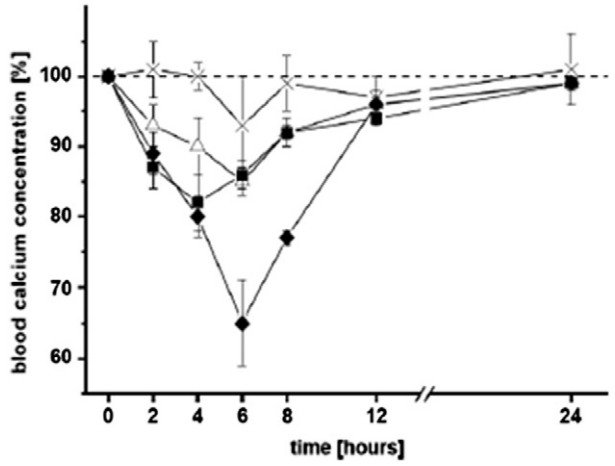

Fig. 11. Blood calcium concentration in rats after oral administration of salmon calcitonin in liposomes coated with preactivated chitosan (□) and thiolated chitosan (■) compared to calcitonin in uncoated liposomes (Δ) and in solution (X).
Adapted from: Gradauer et al. [91].

or sodium hyaluronate, represent the treatment of the first choice. To overcome required frequent instillation, eye drops containing thiomers with stronger mucoadhesive properties were evaluated for their ability to stabilize the precorneal tear film [39]. A study in humans revealed that a solution of thiolated poly(acrylic acid) could prolong the tear film break-up time compared to a commercial product based on carbomer [100]. Within the same indication, the dry eye syndrome, safety of chitosan–N-acetylcysteine eye drops was evaluated on the one hand as single administration with different concentrations of the thiomer and on the other hand after two-times-daily instillation in two phase I clinical trials. Both clinical trials revealed an excellent tolerability [101,102] and eye drops containing chitosan–N-acetylcysteine as lubricant (Lacrimera®; Croma-Pharma) for the therapy of the dry eye syndrome is supposed to be introduced into the European and Canadian markets this year.

6. Conclusion

Immobilization of thiol groups on well-established polymers as well as their S-protection can lead to dramatic improvements in their properties. Ameliorated mucoadhesion, *in situ* gelation, permeation enhancement and efflux pump inhibition qualify this type of multifunctional polymers as promising tools in therapy and drug delivery. These properties have already resulted in the development of numerous different formulations comprising thiomers that show a strongly improved performance over state-of-the-art formulations. Within this year, the first commercial product containing a thiolated polymer (Lacrimera®) will reach the European and Canadian markets providing strong evidence for their potential and inspiring both academia and industry to develop further products utilizing this novel type of polymers.

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