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REVIEW ARTICLE

The Potential of Thiolated Polymers for Peptide Drug Delivery

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ABSTRACT

Thiolated polymers are generated by the immobilisation of thiol-bearing ligands to mucoadhesive polymeric excipients. The mucoadhesive properties of these polymers are improved up to 130-fold by formation of disulfide bonds with mucus glycoproteins. Due to formation of inter and intramolecular disulfide bonds within the thiolated polymer itself, tablets, microparticles or in situ gelling formulations display strong cohesive properties resulting in comparatively higher stability, prolonged disintegration times and a more controlled release of peptide drug. Peptide drug permeation through mucosa can be improved by the use of thiolated polymers. Thiolated polymers also exhibit improved inhibitory properties towards peptidases. The efficacy of thiolated polymers in peptide drug delivery could be demonstrated by various *in vivo* studies. In different studies, formulations comprising the corresponding unmodified polymer had only a marginal or no effect. The results of the studies prove that thiolated polymers have potential for peptide drug delivery.

KEYWORDS: Thiolated polymers, Peptide drug delivery, Mucoadhesion and cohesion, Permeation enhancement, Enzyme inhibition, Controlled drug delivery, Stability of thiolated polymers

INTRODUCTION

The improvement of potent formulations for peptide drug delivery represents one of the main challenges in modern pharmaceutical technology. At present most of these surprising pharmacological potential therapeutic agents have to be administered via parenteral routes, which are inconvenient because of pain, fear and risks being associated with this type of application. 'Injectable-to-non-invasive-conversions' and in particular 'injectable-to-oral-conversions' are consequently highly in demand. In order to provide a sufficiently high bioavailability with non-invasive peptide drug delivery systems, however, various hurdles have to be overcome. They include the diffusion barrier (i) being based on the mucus gel layer covering mucosal membranes, which has to be passed by peptides in order to reach the absorption membrane, and the enzymatic barrier (ii) being represented by secreted and membrane bound peptidases. [1, 2] Moreover, having reached the absorption membrane in intact form therapeutic peptides have to permeate this membrane barrier (iii) in order to reach the systemic circulation. [3] Pharmaceutical technological attempts to overcome these barriers include the use of enzyme inhibitors, permeation enhancers and multifunctional polymers ideally guaranteeing both enzyme inhibition and permeation enhancement. [2, 4, 5] In case of multifunctional polymers these effects, however, can only take place if a tight contact of the polymer with the mucosa is provided for the whole period of peptide drug release and absorption. Apart from enzyme inhibitory and permeation enhancing properties multifunctional polymers should therefore offer strong mucoadhesive features.

Among this group of multifunctional polymers exhibiting all these mentioned properties, thiolated polymers designated thiolated polymers are the most promising for peptide drug delivery. Due to the immobilisation of thiol

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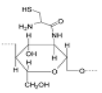
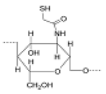
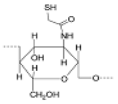
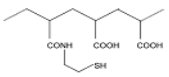
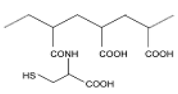
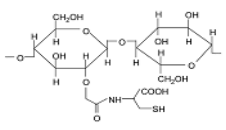
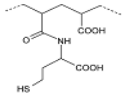
groups on well-established multifunctional polymers such as poly (acrylates) or chitosans their enzyme inhibitory, permeation enhancing and mucoadhesive properties can be strongly improved. [6, 7] Within this review the features of thiolated polymers as well as their advantages and potential for peptide drug delivery are discussed. The summarised data should provide a good starting point for further developments and applications of thiolated polymers in peptide drug delivery.

A. GENERATION OF THIOLATED POLYMERS:

I. Anionic thiolated polymers:

Anionic thiolated polymers generated thus far all exhibit carboxylic acid groups as anionic substructures. These carboxylic acid groups offer the advantage, that sulfhydryl moieties can be easily attached to such polymers via the formation of amide bonds. Appropriate ligands are overall cysteine, cysteamine and homocysteine. [7, 8, 9] The formation of amide bonds can be mediated 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 be almost excluded.

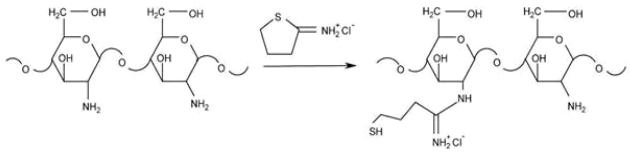
Table 1. Anionic Thiolated Polymers.

Thiolated polymer	Structure	Reference
Chitosan-cysteine		10
Chitosan-thioglycolic acid		11
Chitosan-4-thio-butylamidine		12
Polycarbophil-cysteamine		8
Polycarbophil-cysteine		7
Carboxymethylcellulose-cysteine		13
Poly(acrylic acid)homocysteine		9

addition, disulfide bonds formed during synthesis can be cleaved thereafter by the addition of reducing agents such as dithiothreitol or NaBH4. The total amount of immobilised, reduced and oxidised thiol groups can be determined by reducing first of all the entire amount of oxidised thiol groups with NaBH4 followed by quantifying the thiol groups with Ellman's reagent. Skipping the reduction process allows the determination of the ratio of oxidised to reduced thiol groups. The chemical structure of anionic thiolated polymers is shown in **Table 1. II. Cationic thiolated polymers:**

Cationic thiolated polymers are mainly based on chitosan. The primary amino group at the 2-position of the glucosamine subunits of this polymer is the main target for the immobilisation of thiol groups. As outlined in Fig. 1 sulfhydryl-bearing agents can be covalently attached to this primary amino group via the formation of amide or amidine bonds. In the case of amide bonds the carboxylic acid group of the ligands cysteine and thioglycolic acid react with the primary amino group of chitosan mediated for instance by carbodiimides. [10] The formation of disulfide bonds by air oxidation during the synthesis can be avoided as described above. In the case of amidine bonds 2-iminothiolane is used as coupling reagent. It offers the advantage of a simple one step coupling reaction. In addition, the thiol group of the reagent is protected towards oxidation because of the chitosan with 2-iminothiolane is illustrated in **Fig. 1.** [11]

Fig. 1. A synthetic pathway for the modification of chitosan with 2-iminothiolane. [11]



B. PROPERTIES OF THIOLATED POLYMERS:

I. Mucoadhesive and cohesive properties:

Mucoadhesive properties can provide an intimate contact with the mucosa at the site of drug uptake preventing a presystemic metabolism of peptides on the way to the absorption membrane in the gastrointestinal tract. Additionally, the residence time of the delivery system at the site of drug absorption is increased. Moreover, a steep concentration gradient on the

absorption membrane representing the driving force for passive drug uptake can be provided. Anionic polymers feature mucoadhesive properties via hydrogen bonding, van der Waal's interactions and chain entanglement with the mucus forces stronger than the electrical repulsion caused by electrostatic interactions. [12] In contrast, cationic polymers adhere to the negatively charged mucus mainly due to electrostatic forces. [13] As both anionic and cationic mucoadhesive polymers exhibit a high buffer capacity, a demanded microclimate regarding the pH can be adjusted and maintained over numerous hours within the polymeric network. [14] The strong mucoadhesive properties of thiolated polymers are believed to be based on additional covalent bonds between thiol groups of the thiolated polymers and cysteine-rich subdomains of mucus glycoproteins. [15] This theory was confirmed by findings of mucoadhesion studies, where a higher amount of thiol groups on the polymer resulted in higher mucoadhesive properties. [16, 17, 18] As listed in **Table 2,** the mucoadhesive properties of all polymers thus far tested could be strongly improved by the immobilisation of thiol groups. Among all anionic thiolated polymers, poly (acrylic acid) - cysteine (450 kDa; PAA450-Cys) offers the highest mucoadhesion as determined by tensile studies.

Table 2. Comparison Of The Mucoadhesive Properties of Various Polymeric Excipients.

Polymer	Mucoadhesion work (µJ) means ± s.d. (n=3-8)	Reference
Chitosan HCl	23 ± 10	[21]
Chitosan-TBA	682 ± 100	[25]
Chitosan-thioglycolic acid	234 ± 0.4	[21]
Sodium alginate-cysteine	102 ± 36	[14]
Sodium carboxy methyl cellulose	108 ± 17	[13]
Sodium carboxymethyl cellulose-cysteine	157 ± 6	[13]
Sodium alginate	26 ± 1	[14]
Poly(acrylic acid)	171 ± 53	[24]
Polycarbophil-cysteine	280 ± 68	[7]
Poly(methacrylic acid)-cysteine/starch	90 ± 15	[31]
Poly(methacrylic acid)/starch	28 ± 3	[31]
Polycarbophil	110 ± 28	[7]
Poly(acrylic acid)-cysteine	695 ± 80	[24]

Mucoadhesion studies were performed via tensile tests.

After adjusting this thiolated polymer to pH 3, PAA450-Cys exhibited its strongest mucoadhesion. When the pH of PAA450-Cys was shifted to higher pH levels, mucoadhesion decreased. [22] The chitosan–4-thio-butylamidine conjugate (400 kDa; chitosan–TBA) showed almost 30-fold improved mucoadhesive properties compared to unmodified chitosan. These findings were confirmed by mucoadhesion studies with the rotating cylinder method, where chitosan-TBA-tablets remained attached to porcine intestinal mucosa 130-fold longer than unmodified chitosan tablets. [20] Also for chitosan-TBA the pH of the polymer turned out to be crucial. While conjugates adjusted to pH 3 displayed the strongest mucoadhesion, the mucoadhesive properties of the same polymer were comparatively lower when the pH was adjusted to pH > 5. [20] An explanation for this effect can be given by the pH-dependent reactivity of thiol groups. At pH values above 5, thiol groups become more reactive leading to the formation of disulfide bonds already within the polymeric network itself before reacting with disulfide and/or thiol substructures of the mucus. Thiolated polymers of lower pH are less reactive. Their thiol groups become only reactive when interpenetrating the mucus gel layer exhibiting a pH 5–7. Consequently thiolated polymers of a comparatively lower pH probably form disulfide bonds with the mucus gel layer. Covalent bonds are believed to be formed not only between thiolated polymers and mucus, but also within the thiolated polymers itself. This theory was confirmed by the decrease in free thiol groups within thiolated polymers resulting in an increase in viscosity. [7] Although thiolated polymers show strongly improved mucoadhesive properties, the adhesion of delivery systems being based on such polymers is nevertheless limited by the natural mucus turnover. The mucus turnover in the human intestine, for instance, was determined to be in the range of 12–24h. [23]

II. Permeation enhancing properties:

In order to improve the bioavailability of peptide or protein drugs administered via mucosal routes permeation enhancers often have to be added to the delivery system. Generally, two types of permeation enhancers are in use: low molecular mass permeation enhancers such as sodium salicylate or medium-chain glycerides and polymers displaying permeation enhancing properties. [4, 29] The influence of thiolated polymers on the permeation of hydrophilic model compounds and peptide drugs across freshly excised bovine nasal mucosa, rabbit cornea as

well as intestinal mucosa was evaluated in using type chambers. [25] Various thiolated polymers such as PCP-Cys, CMC-Cys, and chitosan-4-thiobutylamidine showed a strong permeation enhancing effect (**Table 3**). [26] This effect could be further improved due to the addition of the permeation mediator glutathione. [27] As shown in Fig. 3, the transport of rhodamine 123 across freshly excised small intestinal mucosa was significantly improved compared to unmodified chitosan utilising 0.5% chitosan–TBA conjugate combined with 5% glutathione. [20] Results of *in vitro* permeation studies could be confirmed by various *in vivo* studies. The likely mechanism being responsible for the permeation enhancing effect of the thiolated polymers/glutathione system has been ascribed to be based on the inhibition of the enzyme protein tyrosine phosphatase (PTP). PTP is able to dephosphorylate tyrosine residues of occludin, which is believed to play an essential role in the opening process of the tight junctions. This dephosphorylation results in the closing of the tight junctions, leading consequently to a decreased permeation of hydrophilic macromolecules. According to this theory, the inhibition of PTP by reduced glutathione will lead to a phosphorylation and an opening of the tight junctions. However, the inhibitory effect of glutathione is limited as it is rapidly oxidised on the cell surface. [28] The presence of the thiolated polymer is therefore essential, as it prevents the oxidation of glutathione on the surface of the mucosa.

III. Enzyme inhibitory properties:

Presystemic metabolism of peptide and protein drugs in particular in the GI-tract can be regarded as one of the main reasons for their limited bioavailability after administration. Therefore, numerous research groups have focused their interest on the development of drug delivery systems providing a protective effect towards secreted as well as membrane-bound enzymes. Two major strategies have thereby been pursued: the incorporation of low molecular mass enzyme inhibitors and the use of polymers exhibiting enzyme inhibitory properties.

Thiolated polymers are promising candidates within the group of enzyme inhibiting polymers. The inhibitory properties of poly (acrylates) on intestinal proteases were first reported by Hutton *et al.* [29] They found a strong reduction of albumin degradation by a mixture of proteases in the presence of carbomer 934P.

Table 3. Permeation Enhancing Properties Of Thiolated Polymers In Comparison to The Corresponding Unmodified Polymers Tested On Freshly Excised Intestinal Mucosa Of Guinea Pigs.

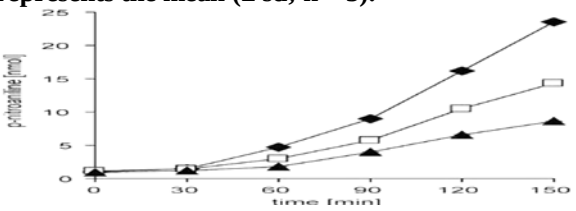
Permeation enhancer	Test compound	Apparent permeability Coefficient [Papp £1026 (cm/s)]	Enhancement ratio (Papp thiolated polymer/Papp unmodified control polymer)	Reference
PCP-Cys	Na-Flu	5.27 ± 0.11	1.57	[38]
PCP-Cys	bac-FITC	2.69 ± 0.09	1.37	[38]
PCP-Cys	insulin-FITC	2.50 ± 0.15	1.35	[38]
PCP-Cys/GSH	Na-Flu	14.64 ± 0.93	2.93	[42]
PCP-Cys/GSH	bac-FITC	9.94±0.82	2.06	[42]
PCP-Cys/GSH	LMWH	0.39 ± 0.02	2.2	[43]
PCP-Cys	LMWH	0.19 ± 0.04	1.1	[43]
PCP-Cys/GSH	hGH-FITC	n.a.	3	[36]
PAA450-Cys	Na-Flu	8.38±0.24	1.29	[39]
PAA450-Cys/GSH	Na-Flu	9.65±0.38	1.48	[39]
PAA-HC/GSH	Na-Flu	5.9 ± 1.97	2.4	[9]
CMC-Cys	Na-Flu	12.92 ± 0.41	1.8	[40]
CMC-Cys	bac-FITC	5.58 ± 0.54	1.32	[40]
CMC-Cys	ins-FITC	5.55 ± 0.65	1.31	[40]
Chitosan-Cys	bac-FITC	not applicable	significant	[10]
Chitosan-TBA/ GSH	rhodamine	3.0 ±1.2	3.6	[30]
Chitosan-TBA	rhodamine	1.5±0.7	1.8	[30]

bac-FITC, fluorescein-isothiocyanate labelled bacithracin; ins-FITC, fluorescein-isothiocyanate labelled insulin; LMWH, low molecular weight heparin; PAA-HC, poly(acrylicacid)-homocysteine; Na-Flu, sodiumfluoresceine; rhodamine, rhodamine 123.

A subsequent study by Lueßen *et al.* [30] showed that PCP and carbomer 934P were potent inhibitors of the proteolytic enzymes trypsin, a-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. [31] PCPCys also had a significantly greater inhibitory effect than unmodified PCP on the activity of isolated aminopeptidase N and aminopeptidase N present on intact intestinal mucosa. [32] The inhibitory effect of thiolated polymers was also tested on intact vaginal mucosa as well as on buccal mucosa. [33] As shown in **Fig. 2**, 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 hereby significantly more effective than the unmodified polymer. The strongly improved enzyme inhibitory properties of PCP-Cys in comparison to unmodified PCP can be explained by the inhibitory effect of L-cysteine itself towards carboxypeptidase A, carboxypeptidase B and aminopeptidase N due to the binding of the Zn^{2+} ion from the enzyme structure. [31, 32]

Fig. 2. Time-course of the formation of p-nitroaniline from leu-pnitroanilide by aminopeptidases present on intact buccal mucosa during incubation without polymer (■); with 0.25% (m/v) polycarbophil (□); with 0.25% (m/v) polycarbophil–cysteine-conjugate (▲). Each point represents the mean (± sd; n = 3). [51]



IV. Thiolated polymers for controlled drug release: A controlled peptide drug release out of the delivery system represents a prerequisite for an increased absorption rate and an enhanced bioavailability. The cohesion and stability of the delivery system over the intended period of peptide drug liberation is in most cases a prerequisite in order to achieve controlled release. Thiolated polymers display excellent cohesive properties. Matrix tablets of PAA450-Cys and PCP-Cys, for instance, were stable for more than 48 h in simulated intestinal fluid without any observable erosion. [19] The thiol functions on the polymeric backbone of thiolated polymers enable them not only to form disulfide bridges with mucus glycoproteins, but also to form inter as well as intramolecular disulfide bonds. This cross-linking of the polymer chains results in the high stability of drug carrier systems based on thiolated polymers.

Thiolated polymers were demonstrated to guarantee a controlled drug release by using model drugs such as fluorescence-labelled insulin. Release studies of fluorescence-labelled insulin showed that an almost zero order release kinetic can be provided by the use of thiolated PCP as carrier matrix. Thiol/disulfide exchange reactions between insulin and the thiolated polymer could thereby be excluded. [34] The reason for this sustained release is the cross-linking within the matrix tablet, which provides a tightened three-dimensional polymeric network leading to a more controlled release. Apart from a sustained peptide drug release over numerous hours a rapid drug release can also be guaranteed, in particular when peptide drugs are incorporated in thiolated polymers microparticles. [35]

V. Stability of thiolated polymers and peptides incorporated in to thiolated polymers:

Because of the sensitivity of thiol groups towards oxidation, the chemical stability of thiolated polymers 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. [36]

Another aspect of stability focuses on the stability of the therapeutic peptide being incorporated in a thiolated polymeric carrier matrix. As most peptide drugs bear thiol and/or disulfide bonds in their chemical structure, thiol/disulfide exchange reactions with thiolated polymers 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 are fulfilled:

- Solid delivery systems with no or comparatively low water content are generated
- The pH of the thiolated polymers 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 thiolated polymers are neighboured by non-ionic or anionic amino acids [31]

Evidence for these theories is not only provided by various *in vitro* studies [31] but also by biofeedback studies in different animal species with different peptide drugs demonstrating that these therapeutic agents do not loose their efficacy having been embedded in a thiolated polymers. [37, 38]

Fig. 3. Permeation enhancing effect of 0.5% (m/v) chitosan–TBA conjugate with 5% (m/v) GSH (■), of 0.5% (m/v) chitosan–TBA (○) and of 0.5% (m/v) unmodified chitosan (▲) on the permeation of rhodamine 123 across freshly excised small intestinal mucosa. Indicated values are means of at least three experiments ±s.d. * Differs unmodified chitosan P, 0:05. [20]

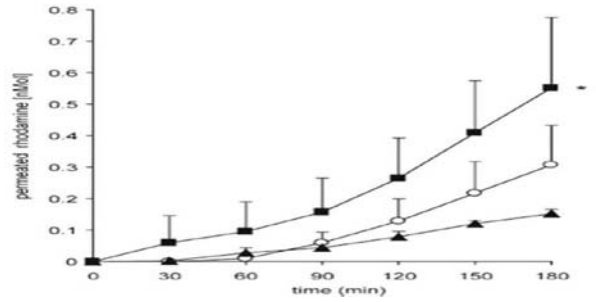
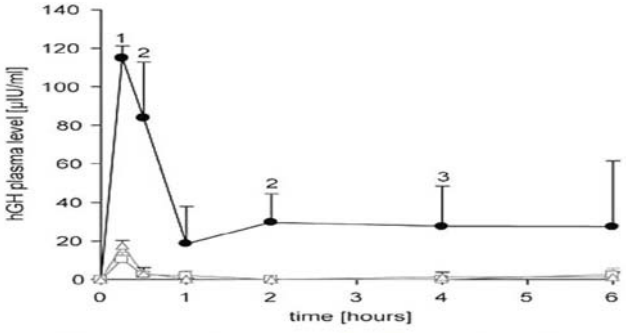


Fig. 4. Plasma concentration-time profiles following intranasal administration of hGH incorporated in PCP-Cys/GSH (●), unmodified PCP (□) and physiological saline (Δ). Data represent the mean ± s.d. of n = 4–5 for all delivery systems. 1 Differs from unmodified PCP control gel P < 0:0001; 2 differs from control P < 0:01; 3 differs from control P <0:05. [52]



C. PEPTIDE DRUG DELIVERY SYSTEMS BASED ON THIOLATED POLYMERS:

I. Tablets:

Tablets are dominant among 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 [38] or in the case of stomach targeted delivery systems, coating with trigylcerides was shown to be sufficient to provide a swelling of the dosage form one it reached the stomach. [37] In addition, thiolated poly (methacrylic acid)/starch compositions were shown to swell only at pH > 5 even without an enteric coating. [21] 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 delivery systems can guarantee a diffusion controlled release.

II. Microparticulate formulations:

Microparticulate formulations based on poly (acrylic acid) or chitosan lack strong cohesive properties. Consequently they disintegrate rapidly and cannot control the release of the embedded peptide drug. Chitosan microparticles can be stabilised by addition of multivalent anions, but as a consequence mucoadhesion decreases. The use of multifunctional polymers like PAA450-Cys for microparticles preparation led to particles with highly improved cohesive properties. [39] They are stabilised by the formation of intramolecular disulfide bonds within the microparticles during the preparation process. Consequently a controlled drug release out of such microparticles can be achieved. The release of the peptide drug can be prolonged by the addition of hydrophobic excipients like Eudragit RS 100 to the polymer. [35] Disintegration studies showed a stability of these thiolated polymeric microparticles over 24 h, whereas particles comprising unmodified poly (acrylic acid) disintegrated within minutes. Microparticles display per se a prolonged residence time on mucosal membranes compared

to single-unit dosage forms. [40] This residence time on mucosal membranes is even further improved when they exhibit mucoadhesive properties. Due to the immobilisation of thiol groups on microparticles the mucoadhesive properties are additionally improved.

III. In situ gelling formulations:

Polymers displaying in situ gelling properties have been described to stabilise themselves once applied as liquid or semisolid formulations at the site of drug delivery. This in situ gelation combines the advantages of a solution, being easy to administer for the patient with the favourable properties of a gel, which displays limited clearance and increased mucoadhesiveness. Several concepts for in situ gelling systems have been described so far. The sol–gel transition can be induced by a shift in pH, in temperature or in electrolyte concentration. [41, 42, 43] Thiolated polymers represent a new type of in situ gelling polymers. [18, 44] 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 interand 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. [45, 46]

D. *IN VIVO* PERFORMANCE-PROOF OF CONCEPT

I. Oral peptide drug delivery:

Although it is impossible to differentiate between the impact of certain properties of thiolated polymers *in vivo*, their overall capability for the oral application of therapeutic peptides could be demonstrated in different *in vivo* studies. [37, 38] The anionic thiolated polymers PCP-Cys and PAA450-Cys were used as drug carrier matrices for insulin and the cationic chitosan–TBA conjugate served as matrix for salmon calcitonin. The mentioned peptide drugs are commercially available as injectable forms and also as a nasal spray in the case of calcitonin. However, patient compliance for these dosage forms is low due to the inconvenient form of application. In contrast, oral administration would improve patient compliance dramatically, but until now oral bioavailability has been too low to permit therapeutic employment. [47] Therefore the drugs mentioned above were chosen as model candidates to evaluate the potential of thiolated polymers as carrier systems for the peroral administration of peptides.

Guggi *et al*. evaluated chitosan-TBA conjugate tablets comprising calcitonin. Small intestine targeted tablets and stomach targeted formulations were tested. The drug delivery systems based on chitosan–TBA-conjugate contained salmon calcitonin, optionally the permeation mediator, reduced glutathione, and different enzyme inhibitors to avoid enzymatic degradation. Chitosan-BBI and chitosan-elastatinal conjugate were added to enteric coated delivery systems targeted to the small intestine. The stomach targeted calcitonin delivery system comprised a chitosan-pepstatin A conjugate to inhibit pepsinic degradation of the protein. The different calcitonin delivery systems were orally administered to rats and the plasma calcium level as pharmacological response was determined. The oral application of calcitonin in ascorbic acid solution and control tablets based on unmodified chitosan resulted in no significant effect. In contrast, calcitonin in chitosan-TBA conjugate tablets led to a more than 5% decrease of the plasma calcium level. Thiolated chitosan tablets comprising reduced glutathione displayed a significantly higher pharmacological efficacy compared to chitosan-TBA tablets lacking this permeation mediator. The strongest effect was achieved with the stomach targeted system. The calcium level decreased by more than 10% and the effect lasted for more than 12 h. [37]

II. Nasal peptide drug delivery:

The nasal route represents an attractive alternative to parenteral delivery for an increasing number of therapeutic peptides such as calcitonin, insulin, desmopressin, buserelin and octreotide. However, bioavailabilities of nasally administered peptides often do not exceed 1% due to low membrane permeability, a short local residence time at the site of absorption and a high metabolic turnover in the nasal epithelium [48] The three major strategies to increase the bioavailability of intranasally administered peptide drugs are (i) the use of permeation enhancers, (ii) incorporation of enzyme inhibitors and (iii) increasing local drug residence time using mucoadhesive polymers. [49] Thiolated polymers seem to be capable of combining most of these strategies. Therefore, the suitability of thiolated polymers as multifunctional

vehicles for systemic nasal peptide drug delivery was evaluated *in vivo*. As model drug, human growth hormone (hGH), a protein drug of 191 amino acids (22 kDa) was utilised, which is used to treat short stature in children due to growth hormone deficiency, Turner's syndrome or chronic renal failure. Currently, hGH has to be administered by daily injections, which are difficult and painful resulting n low patient acceptance. [50] A nasal delivery system for hGH would therefore be highly appreciable.

For the *in vivo* study, an aqueous nasal gel formulation was developed consisting of PCP-Cys, glutathione and hGH in a final concentration of 0.3, 0.5 and 0.6% (m/v), respectively. As controls a 0.3% (m/v) PCP gel and physiological saline were prepared containing the same amount of hGH. These formulations were administered to conscious rats (n=4-5) and the hGH plasma level as monitored via ELISA as a function of time. As shown in Fig. 4, the PCP-Cys/glutathione/hGH nasal gel delivery system resulted in a significantly higher hGH plasma concentration compared to both controls with an absolute bioavailability of 2.75 ± 0.37%. Furthermore, in contrast to the controls the thiolated polymers gel delivery system was able to prolong the efficacy of hGH.

CONCLUSION

The immobilisation of thiol-bearing compounds on polymeric excipients such as poly(acrylates) and chitosans leads to a significant improvement in their mucoadhesive, permeation enhancing and enzyme inhibitory properties. As the cohesive properties are also strongly improved because of a cross-linking process via disulfide bond formation within the polymeric network, a mainly diffusion-controlled sustained release of thiolated polymers embedded peptide drugs can be guaranteed. In comparison to peptide drug delivery systems comprising unthiolated multifunctional polymers, the efficacy of delivery systems comprising the corresponding thiolated version is therefore significantly higher. A 'proof of concept' could meanwhile be provided in various animal species, for various routes of application using various therapeutic peptides embedded in different types of thiomers. According to these results, thiomers seem to represent excellent potential containing polymers for peptide drug delivery.

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