Expert Opinion

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Thiomers for oral delivery of hydrophilic macromolecular drugs

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In recent years thiolated polymers (thiomers) have appeared as a promising new tool in oral drug delivery. Thiomers are obtained by the immobilisation of thio-bearing ligands to mucoadhesive polymeric excipients. By the formation of disulfide bonds with mucus glycoproteins, the mucoadhesive properties of thiomers are up to 130-fold improved compared with the corresponding unmodified polymers. Owing to the formation of inter- and intramolecular disulfide bonds within the thiomer itself, matrix tablets and particulate delivery systems show strong cohesive properties, resulting in comparatively higher stability, prolonged disintegration times and a more controlled drug release. The permeation of hydrophilic macromolecular drugs through the gastrointestinal (GI) mucosa can be improved by the use of thiomers. Furthermore, some thiomers exhibit improved inhibitory properties towards GI peptidases. The efficacy of thiomers in oral drug delivery has been demonstrated by various *in vivo* studies. A pharmacological efficacy of 1%, for example, was achieved in rats by oral administration of calcitonin tablets comprising a thiomer. Furthermore, tablets comprising a thiomer and pegylated insulin resulted in a pharmacological efficacy of 7% after oral application to diabetic mice. Low-molecular-weight heparin embedded in thiolated polycarbophil led to an absolute bioavailability of ≥ 20% after oral administration to rats. In these studies, formulations comprising the corresponding unmodified polymer had only a marginal or no effect. These results indicate drug carrier systems based on thiomers appear to be a promising tool for oral delivery of hydrophilic macromolecular drugs.

Keywords: controlled release, enzyme inhibition, mucoadhesion, oral drug delivery, permeation enhancement, thiomers

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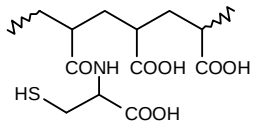
1. Introduction

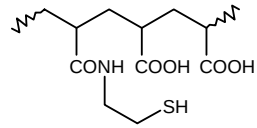
Oral delivery of hydrophilic macromolecular drugs has become one of the major challenges in modern pharmaceutical technology. At present, most of these drugs have to be administered via parenteral routes, which are inconvenient because of the pain, fear and risks associated with this type of application. 'Injectable-to-non-invasive-conversions' and, in particular, 'injectable-to-oral-conversions' are consequently of high demand. However, in order to provide a sufficiently high bioavailability for oral delivery systems, various barriers have to be overcome. These include the diffusion barrier (mucus gel layer covering mucosal membranes), which has to be passed by hydrophilic macromolecular drugs to reach the absorption membrane [1], and the enzymatic barrier, for example, secreted and membrane-bound peptidases in case of peptide drugs [2]. Furthermore, having reached the absorption membrane intact, therapeutic macromolecules must permeate this membrane barrier in order to reach the systemic circulation [3].

Strategies to overcome these barriers include: the use of enzyme inhibitors [2]; permeation enhancers [4]; and multifunctional polymers [5], ideally guaranteeing both

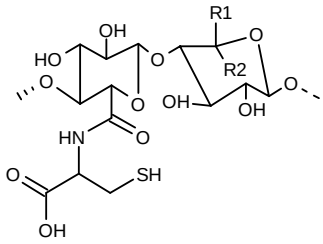
Anionic thiomers



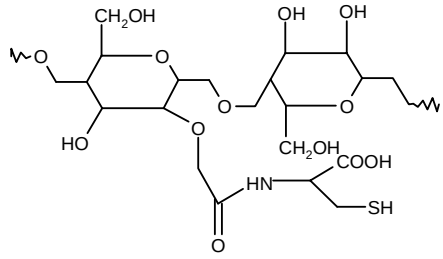
Polycarbophil–cysteine [7]



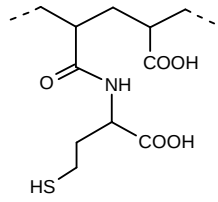
Polycarbophil–cysteamine [8]



Alginate–cysteine (R1=H, R2=COOH or R1=COOH, R2=H) [15]

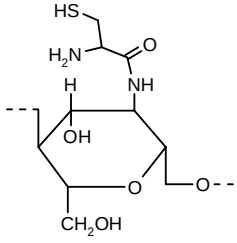


Carboxymethylcellulose–cysteine [14]

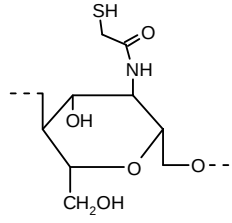


Poly(acrylic acid)–homocysteine [9]

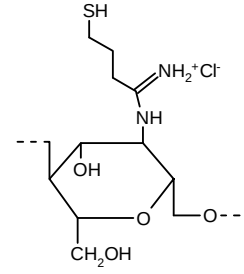
Cationic thiomers



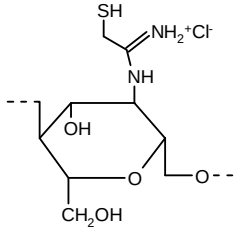
Chitosan–cysteine [13]



Chitosan–thioglycolic acid [11]



Chitosan–4-thio-butylamidine [12]



Chitosan–thioethylamidine [10]

Figure 1. Structure of thiolated polymers.

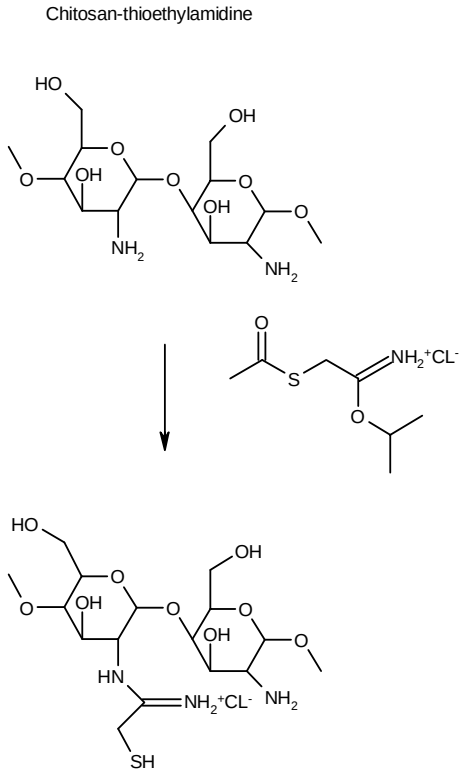


Figure 2. Synthetic pathway for the modification of chitosan with isopropyl-*S*-acetylthioacetimidate·HCl. Adapted from Kafedjiiski *et al.* [10].

enzyme inhibition and permeation enhancement. However, in case of multifunctional polymers, these effects can only take place if a tight contact of the polymer with the gastrointestinal (GI) mucosa is provided, ideally for the whole period of drug release and absorption. Multifunctional polymers should, therefore, also offer strong mucoadhesive features.

Among multifunctional polymers exhibiting the above properties, thiolated polymers (thiomers) appear to be the most promising for oral macromolecule delivery. Due to the immobilisation of thiol groups on well-established multifunctional polymers, such as chitosans or poly(acrylates), their permeation-enhancing, enzyme inhibitory and mucoadhesive properties are strongly improved [6,7]. Within this review the features of thiomers, as well as their advantages and potential for oral delivery, are discussed. The summarised data provides a good starting point for further developments and applications of thiomers in oral drug delivery.

2. Synthesis of thiomers

2.1. Anionic thiomers

So far, developed anionic thiomers exhibit all carboxylic acid groups as anionic substructures. These carboxylic acid groups offer the advantage that sulfhydryl compounds, bearing a primary amino group, can be easily attached to such polymers via the formation of amide bonds. At present, the ligands used are cysteine (Cys) [7], cysteamine [8] and homocysteine [9]. The formation of amide bonds can be mediated by carbodiimides. Performing the reaction under inert conditions allows the exclusion of an unintended oxidation of thiol groups during synthesis. Alternatively, the synthesis can be performed at a pH of < 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 avoided. Furthermore, disulfide bonds formed during synthesis can be cleaved thereafter by the addition of reducing agents, such as dithiothreitol, $NaBH_4$ or tris-(2-carboxyethyl)-phosphine hydrochloride. The total amount of immobilised oxidised and reduced thiol groups can be determined by reducing first the entire amount of oxidised thiol groups with $NaBH_4$, followed by quantifying the thiol groups with Ellman's reagent. Skipping the reduction process allows the ratio of reduced to oxidised thiol groups to be determined. The chemical structures of anionic thiomers generated so far, are shown in Figure 1 [7-14].

2.2. Cationic thiomers

The most important polymer for the development of cationic thiomers is chitosan. The primary amino group at the C2 of the glucosamine subunits is the main target for the immobilisation of thiol groups. Sulfhydryl-bearing agents can be covalently attached to this primary amino group via the formation of amide or amidine bonds (Figure 1). In terms of amide bonds, the carboxylic acid group of thioglycolic acid and Cys ligands, reacts with the primary amino group of chitosan mediated by carbodiimides. Disulfide bond formation caused by air oxidation during the synthesis can be avoided, as described above. The reactivity of amino groups, however, decreases at lower pH values by controlling the oxidation of thiol groups, and, therefore, resulting in less modification. An undesired side-reaction with Cys ligands is the carbodiimide-mediated formation of Cys–Cys side chains.

In case of the formation of amidine bonds, 2-iminothiolane can be used as coupling reagent, yielding chitosan–4-thiobutylamidine (chitosan–TBA) [101]. This offers the advantage of a simple, one-step, coupling reaction. In addition, the thiol group of the reagent is protected from oxidation because of the chemical structure of the reagent.

Storage studies, under N_2, have shown that the content of free thiol moieties decreases slightly over time, and might be associated with an undesired recyclisation reaction [10]. This side-reaction may occur after the derivatisation of different amines with 2-iminothiolane, involving the loss of ammonia and yielding *N*-substituted 2-iminothiolanes. Thus, a ligand possessing only one methylene group, instead of three, would be favourable, as recyclisation cannot occur due to reduced chain length. Therefore, chitosan was modified with isopropyl-*S*-acetylthioacetimidate·HCl, yielding chitosan–thioethylamidine [101], (Figure 2). This reagent also features a

Table 1. Permeation-enhancing properties of thiomers in comparison with the corresponding unmodified polymers tested on freshly excised intestinal mucosa of guinea-pigs.

Permeation enhancer	Test compound	Enhancement ratio (P_{app} thiomer/P_{app} unmodified control polymer)	Apparent permeability coefficient (P_{app} 10^{-6} [cm/s])	Ref.
Chitosan–TBA	Rhodamine	1.8	1.5 ± 0.7	[22]
Chitosan–TBA/GSH	Rhodamine	3.6	3.0 ± 1.2	[22]
Chitosan–Cys	bac–FITC	Significant	NA	[13]
PAA–HC/GSH	NaFlu	2.4	5.9 ± 1.97	[9]
PAA_{450}–Cys/GSH	NaFlu	1.48	9.65 ± 0.38	[23]
PAA_{450}–Cys	NaFlu	1.29	8.38 ± 0.24	[23]
PCP–Cys/GSH	hGH–FITC	3	NA	[24]
PCP–Cys	LMWH	1.1	0.19 ± 0.04	[25]
PCP–Cys/GSH	LMWH	2.2	0.39 ± 0.02	[25]
PCP–Cys/GSH	bac–FITC	2.06	9.94 ± 0.82	[26]
PCP–Cys/GSH	NaFlu	2.93	14.64 ± 0.93	[26]
PCP–Cys	Insulin–FITC	1.35	2.50 ± 0.15	[18]
PCP–Cys	bac–FITC	1.37	2.69 ± 0.09	[18]
PCP–Cys	NaFlu	1.57	5.27 ± 0.11	[18]

bac–FITC: Fluorescein–isothiocyanate-labelled bacithracin; GSH: Glutathione; hGH–FITC: Fluorescein–isothiocyanate-labelled human growth hormone; Insulin–FITC: Fluorescein–isothiocyanate labelled insulin; LMWH: Low-molecular-weight heparin; NaFlu: Sodium fluorescein; PAA–HC: Poly(acrylic acid)–homocysteine; P_{app}: Apparent permeability coefficient; PCP: Polycarbophil; Rhodamine: Rhodamine-123; TBA: 4-Thiobutylamidine.

sulfhydryl-protecting group, which is cleaved in the course of the reaction [10]. The chemical structures of cationic thiolated polymers generated so far are also shown in Figure 1.

3. Advantages of thiomers

3.1 Permeation-enhancing properties

Attempts to overcome the absorption barrier are mainly based on the co-administration of permeation enhancers, such as low-molecular-mass permeation enhancers [102] and polymeric permeation enhancers [103]. Various classes of small molecules have proven to be useful in improving the permeation across intact epithelial membranes, such as sodium salicylate, Na_2 EDTA, sodium caprate and phospholipids [4,16,104]. Some of them, however, can cause intestinal damage, or can even enter the systemic circulation due to their low molecular mass, leading to systemic toxic effects.

Another class of permeation enhancers that has recently gained a lot of attention are thiomers. They display some advantages over small-molecular enhancers, such as additionally strong mucoadhesive properties, which allow them to remain concentrated at the area of drug absorption. Furthermore, as high-molecular-mass polymers will not be absorbed from the mucosal barriers, systemic side effects can be excluded [17]. Various thiomers, such as polycarbophil–Cys [18], poly(acrylic acid) (PAA)–Cys (MW 450 kDa) [19], carboxymethylcellulose–Cys [20], chitosan–Cys [13] and chitosan–TBA [21] show a strong permeation-enhancing effect on model compounds *in vitro*. Results of these studies are shown in Table 1. The combination of thiomer with reduced glutathione (GSH) led to a significant improvement of the enhancement ratio [26]. For example, the system of polycarbophil–Cys (0.5%) with GSH (0.4%) led to an enhancement ratio ≤ 2.93 for the model substance sodium fluorescein. By increasing the amount of immobilised Cys, a higher uptake of sodium fluorescein was achieved, as shown in Figure 3. In another study, 0.5% chitosan–TBA combined with 5% GSH, showed a pronounced permeation enhancing effect in comparison with unmodified chitosan. The uptake of the cationic marker compound, rhodamine-123, was threefold higher in the presence of thiolated chitosan versus unmodified chitosan [22].

The mechanism thought to be responsible for this improved permeation enhancing effect is the inhibition of the enzyme protein tyrosine phosphatase (PTP). PTP is responsible for the dephosphorylation of tyrosine subunits of occludin, which is involved in the opening process of tight junctions. When these tyrosine subunits of occludin are dephosphorylated, the tight junctions are closed. In contrast, when they are phosphorylated, the tight junctions are opened. The inhibition of PTP by compounds such as reduced GSH consequently leads to a phosphorylation and the opening of tight junctions. However, the inhibitory effect of GSH is limited as it is rapidly oxidised on the mucosal surface [27]. Thus,

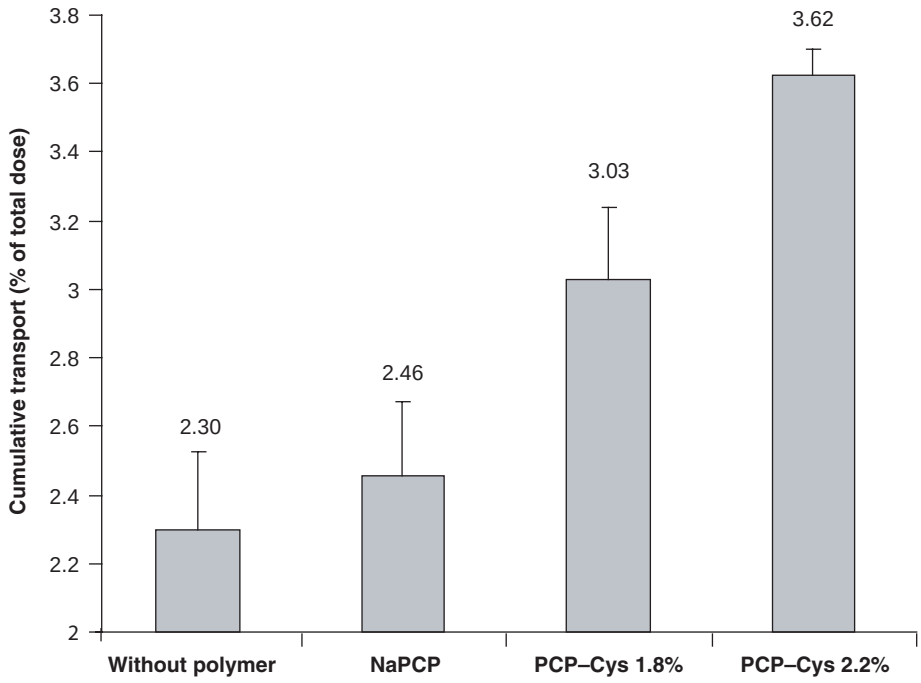


Figure 3. Comparison of the effect of PCP derivatives with increasing amounts of covalently attached Cys (PCP–Cys) on the cumulative transport of sodium fluorescein across the small intestinal mucosa of guinea-pigs at pH 7.4 and 37°C. Each point represents the mean ± s.d. of three experiments. Adapted from Clausen *et al*. [18].
PCP: Polycarbophil.

the presence of the thiolated polymer is essential, as it prevents the oxidation of GSH on the surface of the mucosa.

In addition, the high efficacy of thiomers to enhance mucosal uptake has been shown in various *in vivo* studies. Calceti *et al*. [28], for example, gained a pharmacological efficacy of 7% of orally applied pegylated insulin in diabetic mice by incorporating the peptide in a minitablet based on a PAA–Cys conjugate containing 2% GSH.

3.2 Enzyme inhibitory properties

The enzymatic degradation of orally-administered peptide drugs in the GI tract can be regarded as one of the main reasons for the poor bioavailability of this type of hydrophilic macromolecular drug. Hence, 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 [105]. Two major strategies have thereby been pursued: the addition of low-molecular-mass enzyme inhibitors, and the use of polymers showing enzyme-inhibitory properties (chitosan–EDTA conjugates) [29]. As low-molecular-mass enzyme inhibitors are extensively diluted in the GI tract and, in many cases, faster absorbed than the peptide drug itself, their efficacy remains questionable. In addition, various systemic toxic side effects as well as feedback regulations, leading to an increased enzymatic activity cannot be excluded. Polymers do not seem to have these drawbacks.

Thiomers 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.* [30]. Hutton found a strong reduction of albumin degradation by a mixture of proteases in the presence of carbomer 934P. A subsequent study by Lueßen *et al*. [31] showed that polycarbophil and carbomer 934P were potent inhibitors of the proteolytic enzymes trypsin, α-chymotrypsin and carboxypeptidase A. By the covalent attachment of Cys to polycarbophil, the inhibitory effect of the polymer towards carboxypeptidase A, carboxypeptidase B and chymotrypsin could be significantly improved [32]. Thiolated polycarbophil also had a significantly greater inhibitory effect than unmodified polycarbophil, on the activity of isolated aminopeptidase-*N* and on aminopeptidase-*N* present on intact intestinal mucosa [33].

The strongly improved enzyme inhibitory properties of thiolated polycarbophil in comparison with unmodified polycarbophil can be explained by the inhibitory effect of L-Cys itself towards carboxypeptidase A, carboxypeptidase B and aminopeptidase-*N*, due to the binding of Zn^{2+} from the enzyme structure [32,33].

3.3 Mucoadhesive and cohesive properties

Balanced adhesive and cohesive properties of multifunctional polymers are essential for their efficacy in oral drug delivery systems, especially concerning mucoadhesive tablets and microparticles. Mucoadhesive drug delivery systems should provide an intimate contact for the drug with the mucosa over an extended period of time. Thus, a steep

Table 2. Comparison of the mucoadhesive properties of various polymeric excipients. Mucoadhesion studies were performed via rotating cylinder method.

Polymer	Degree of modification (µmol/g)	Adhesion time (h)	Improvement ratio	Ref.
Chitosan–TBA	682	> 160	> 94	[22]
PAA–Cys	695	22	13	[39]
Chitosan–TEA	140	24	8.9	[10]
Chitosan–thioglycolic acid	27	4	5	[37]
Polycarbophil–Cys	12	> 10	2.1	[14]
Sodium carboxymethylcellulose–Cys	22	3	1.2	[14]

Improvement ratio = adhesion time of thiomer/adhesion time of corresponding unmodified polymer.
PAA: Poly(acrylic acid); TBA: 4-Thiobutylamidine; TEA: Thioethylamidine.

concentration gradient of the drug towards the absorption membrane and consequently higher bioavailability can be achieved. Because of the great potential of this concept various patents have been filed focusing on mucoadhesive delivery systems [106,107].

Many theories have been proposed to describe mucoadhesion such as the electronic, adsorption, wetting and diffusion theory. Although the exact mechanism of anionic polymer adhesion has not been thoroughly investigated, a key part is the secondary molecular interaction with the mucus via van der Waal's forces and hydrogen bonding [34]. Cationic polymers adhere to the negatively-charged mucus, mainly due to electrostatic forces [35]. In the case of thiomers, mucoadhesion occurs by the formation of additional covalent bonds between thiol groups of thiomers and Cys-rich subdomains of mucus glycoproteins [36]. Thus, the mucoadhesive properties of thiomers are strongly improved. This theory was confirmed by the results of tensile studies of thiomers, which demonstrated a positive correlation between the degree of modification with thiol-bearing moieties and the adhesive properties (the total work of adhesion [TWA]) of the polymer [37,38].

The adhesion time of the thiolated conjugates also increased by increasing the amount of immobilised thiol groups (Table 2). Polymers of chitosan–TBA and chitosan–thioetylamidine conjugates exhibit additionally increased mucoadhesive properties due to improved ionic interactions between the cationic amidine substructure of the conjugates and anionic substructures within the mucus layer.

Among all anionic thiomers, PAA_{450}–Cys (with a molecular mass of 450 kDa) conjugate displayed the strongest mucoadhesive properties. The thiolated PAA_{450} polymer exhibited the highest TWA determined so far, in comparison with other thiolated polymers tested under the same conditions [40].

The polymer chain length, expressed by the molecular mass, also influences the mucoadhesive properties of the polymer. PAA–Cys conjugates of lower molecular mass (2, 45, 250 kDa) exhibit minor mucoadhesive properties. Polycarbophil–Cys showed more than twofold less mucoadhesive properties compared with the PAA_{450}–Cys conjugate, probably due to reduced chain flexibility [39]. The medium molecular mass of chitosan–TBA (400 kDa) had a fourfold improved adhesion, evaluated with the rotating cylinder method, in comparison with the derivative of low-molecular-mass [41].

Moreover, results demonstrated that the mucoadhesiveness of PAA_{450}–Cys conjugates can be increased by adjusting the pH to lower levels (pH 3) [40]. Chitosan–TBA conjugates adjusted to pH 3 also displayed the strongest mucoadhesion. When the pH of the polymer was shifted to higher pH-levels, the mucoadhesion decreased [22]. An explanation for this effect can be given by the pH-dependent reactivity of thiol groups. At pH values of ≥ 5, thiol groups become more reactive, which leads to the formation of disulfide bonds already within the polymeric network, before reacting with thio-substructures of the mucus. Consequently, the lower the pH of the thiomer, the less reactive the thiol groups. Only thiol groups coming into direct contact with the mucus gel layer will be activated as a consequence of a shift to pH 5 – 7.

The disintegration behaviour of thiomers compressed into tablets is a good indicator for their cohesive features. According to *European Pharmacopeia*, disintegration studies of PAA–Cys tablets demonstrated that the disintegration time increases with increasing molecular mass of the polymers. In particular, matrix tablets of PAA–Cys and polycarbophil–Cys were stable for > 2 days and no erosion was observed over this time [39]. PAA–Cys conjugate tablets were also significantly more stable, with a further increase in stability at higher pH values (pH 6 – 8). Thiolated polymer tablets showed a 3 – 20-fold more prolonged disintegration time than unmodified poly(acrylic) acid tablets. This can be explained by the formation of stabilising disulfide bonds within the polymeric network, which is favoured at higher pH [40]. This theory was confirmed by the decrease in free thiol groups within thiomers during the oxidation experiments, resulting in an increase in viscosity [7,15].

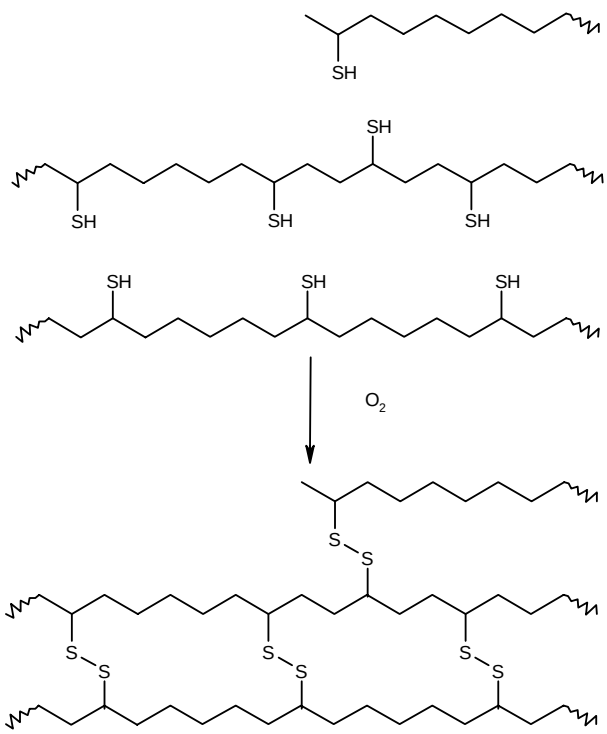


Figure 4. Oxidation of thiol groups onto the thiomer matrix tablet.

3.4 Thiomers as matrices for controlled drug release

Controlled release of the drug out of polymeric carrier systems is, in many cases, essential for an increased absorption rate and an enhanced oral bioavailability. In addition, the cohesion and stability of the polymeric network, over the intended period of drug liberation, is in most cases a prerequisite to achieve controlled release.

Thiomers show comparatively high cohesive properties. Matrix tablets of thiolated PAA and thiolated polycarbophil, for example, are stable for ≥ 48 h in simulated intestinal fluid, without any observable erosion [39]. The thiol functions of thiomers enable them to form inter- and intramolecular disulfide bonds. This crosslinking of the thiomer chains results in the high stability of the polymeric network.

Thiomers used as a drug carrier matrix have been demonstrated to provide an almost zero-order release kinetic for model drugs, such as fluorescence-labelled insulin [42]. The reason for this sustained release is the crosslinking within the matrix tablet, which guarantees a tightened three-dimensional polymeric network, and a more controlled release. Thiol/disulfide exchange reactions between insulin and the thiolated polymer could thereby be excluded [42]. Apart from a sustained release of hydrophilic macromolecular drugs over numerous hours, a rapid drug release can also be guaranteed within 30 min, in particular when the therapeutic agent is incorporated in thiomer microparticles or nanoparticles [43].

3.5 Thiomer–drug interactions

For most hydrophilic macromolecular drugs, such as nucleic acids or heparins, interactions with thiomers can be excluded. With peptide drugs bearing thiol and/or disulfide groups, however, thiol/disulfide exchange reactions concerning thiomers cannot be excluded. Studies investigating such peptide–thiomer interactions revealed that they take place only to a limited extent. For the majority of therapeutic peptides such interactions can be excluded. Generally, thiol/disulfide exchange reactions do not seem to take place in most cases if at least one of the following demands are fulfilled:

- solid dosage forms with no, or comparatively low, water content are generated
- the pH of the hydrated drug carrier matrix, being based on a thiomer, is ≤ 5, resulting in 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 embedded in a cationic thiomer are neighboured by non-ionic or cationic amino acids [32]

These theories are based on various *in vitro* [32] and biofeedback studies, in different animal species with different peptide drugs, showing that these therapeutic agents do not loose their efficacy when they have been embedded in a thiomer [44,45].

4. Thiomer delivery systems

4.1 Tablets

The most commonly used dosage forms for versatile pharmaceutical preparations are tablets, which are orally administered and are available in various forms. This dosage form has multiple advantages, as tablets are cost-effective to manufacture, convenient to dispense, easy for the patient to administer, and provide a versatile means of delivering the drug. The release of drug from the tablet can be controlled by altering the design and composition of the formulation.

In the most appropriate model, a tablet is described as powder particles dispersed in air, resulting in a tablet containing a network of pores [46]. As a consequence of this model, the interparticulate bonds are considered weaker than the intraparticulate bonds. The predominant bonding mechanisms between particles in compacts are divided into three main types: distance forces; solid bridges; and mechanical interlocking. In the case of thiomer tablets, the oxidation of thiol groups and the formation of disulfide bonds already takes place within the matrix tablet (Figure 4). It might be suggested for the thiomer tablets that the predominant bonding mechanism is similar to the solid bridges, built through interparticulate disulfide bonds. This mechanism explains the disintegration behaviour of the thiomer tablets, particularly the extended disintegration time. Tablets consisting of PAA_{450}–Cys, for example, showed a prolonged disintegration of ≥ 48 h [39]. In order to characterise the oxidation process of thiol groups to disulfide bonds within matrix tablets, the

degree of oxidation has been quantified as a function of time in aqueous solutions [40]. The amount of thiol groups within the thiomer tablets remained ~ 80% from the initial content after 5 h of incubation.

The crosslinking process might provide a tightened three-dimensional polymeric network, leading to a more controlled drug release. The crosslinking process can be controlled by adjusting the polymer solution to a defined pH before lyophilisation [40] and by adding a certain amount of sugar alcohols, such as mannitol, to the tablet composition [42].

Mixing poly(methacrylic) acid (PMAA) and starch in acidic aqueous solutions leads to complexes, which can be used as carrier matrix for various drugs. PMAA–starch compositions can guarantee insolubility of dosage forms in the gastric fluid. They provide a controlled drug release at a pH of ≥ 5 and display a protective effect of incorporated drugs towards hydrolytic degradation in the acidic milieu. Furthermore, a pepsinic degradation of embedded peptides can be excluded [47]. Thiolated PMAA–starch compositions are further developed to provide better mucoadhesion [48]. The resulting thiolated PMAA–starch composition consists of 24% thiolated PMAA and 76% starch. The mucoadhesive properties of the thiolated PMAA–starch and unmodified PMAA–starch were evaluated by using two different test methods. Tensile studies showed a threefold improvement in the total work of adhesion compared with an unmodified PMAA–starch composition. These results were confirmed by the rotating cylinder method. The thiolated PMAA–starch composition exhibited a threefold improvement in the adhesion time over the unmodified composition.

If polymers are used as drug carrier matrices for tablets, the polymer forms a gel following contact with the liquids, such as that of the mucosal membranes. To guarantee a swelling of orally administered tablets right on the intestinal mucosa, tablets can be enteric coated [45], or, in the case of stomach-targeted delivery, coating tablets with triglycerides has been shown to be sufficient to provide a swelling of the dosage form, once it has reached the stomach [44].

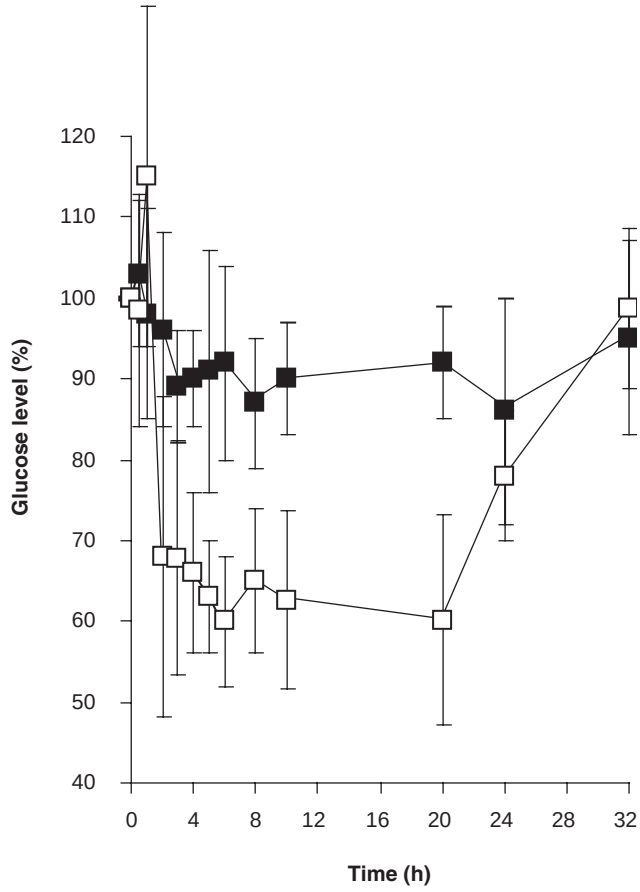


Figure 5. Decrease in blood glucose level in diabetic mice after oral administration of pegylated insulin loaded tablets (closed squares) and pegylated insulin in solution (open squares). Each point represents the mean ± s.d. of 10 experiments. Adapted from Calceti *et al.* [28].

4.2. Microparticles

Although a number of mucoadhesive microparticulate formulations for local or systemic drug delivery have been described in the literature, clinical application is still limited. Thus, there remains a need for the development of efficient delivery systems that combine the advantage of mucoadhesion with that of controlled drug delivery. The solvent evaporation process is frequently used to encapsulate drugs into microparticles. It is well known that the drug candidate must be soluble in the organic phase. When it is highly hydrophilic, the water/water-in-oil (w/ow)-multiple emulsion method is particularly suitable for encapsulation.

Microparticles based on PAA or chitosan lack strong cohesive properties. Hence, they disintegrate rapidly and cannot control the release of the embedded peptide drug. Chitosan microparticles can be stabilised by adding multivalent anions, but, as a result, mucoadhesion decreases. Chitosan microparticles were prepared by dropping a chitosan solution into sodium tripolyphosphate, through ionic crosslinking. Tensile tests showed that all microspheres were capable of adhering to porcine oesophageal mucosa, as the particles prepared from the PAA exhibited greater mucoadhesive strength than those produced with chitosan [49].

The use of multifunctional thiomers such as PAA_{450}–Cys for microparticle preparation has led to particles with highly improved cohesive properties. Microparticles were prepared by a w/o emulsification solvent evaporation technique. Results demonstrated that the higher the pH of the aqueous phase during the preparation process, the higher the degree of crosslinking within the particles. Because of the formation of disulfide bonds within the particles, they did not disintegrate under physiological conditions within 48 h. In addition, a controlled release of bromelain was achieved. The mucoadhesive properties of the thiolated microparticulate systems were improved threefold, in comparison with the particles

comprising unmodified PAA. By the addition of hydrophobic excipients, such as Eudragit® RS (Röhm GmbH & Co.) to the polymer, the release of the peptide drug can be prolonged [43]. The higher the ratio of Eudragit RS in the microparticles, the more prolonged the release of insulin. Disintegration studies showed a stability of these thiomeric microparticles over 24 h, whereas particles comprising unmodified PAA disintegrated within minutes.

Particulate delivery systems display a prolonged residence time on mucosal membranes compared with single-unit dosage forms [50]. The residence time on these membranes is further improved when microparticles exhibit mucoadhesive properties. Because of the immobilisation of thiol groups on microparticles, the mucoadhesive properties are additionally improved. PAA_{450}–Cys microparticles, for example, were almost 14-times more mucoadhesive on the intestinal mucosa than unmodified polymer particles [43].

5. *In vivo* studies

5.1. Oral delivery of insulin

The development of an oral insulin formulation is perhaps one of the greatest challenges in oral drug delivery. Marschütz *et al.* [45] developed insulin tablets based on the thiolated polymer polycarbophil–Cys, containing the enzyme inhibitors elastatinal and Bowman-Birk-inhibitor covalently linked to carboxymethylcellulose. This insulin formulation led to a maximum decrease of the blood glucose level of 36%, and the effect held for ≥ 80 h.

In another study, Calceti *et al.* [28] generated insulin tablets with PAA_{450}–Cys as the drug carrier matrix. Insulin was chemically modified with poly(ethylene glycol) in order to achieve a higher stability towards elastase. After oral administration to diabetic mice these tablets led to a decrease of the blood glucose level of ~ 60% and the effect lasted for 20 h. Orally administered pegylated insulin in solution did not show an effect at all. The pharmacological efficacy of this oral formulation was calculated to be 7% compared with subcutaneous injection. Results of this study are shown in Figure 5 [28].

5.2. Oral delivery of calcitonin

At present, calcitonin is administered via either the nasal or parenteral route. An oral delivery system for this peptide drug is, therefore, in high demand [51]. Calcitonin is very poorly absorbed through the GI tract and significant proteolytic degradation takes place in the intestinal lumen, which limits its clinical usefulness. Several approaches have been utilised to overcome these limitations, but limited success has been achieved. Using permeation enhancers of relatively low molecular mass, significant oral uptake of salmon calcitonin was achieved [52].

Guggi *et al.* [44] evaluated calcitonin tablets comprising a thiolated chitosan. Small intestine- and stomach-targeting formulations were generated. The delivery systems contained salmon calcitonin, optionally the permeation-mediator reduced GSH and different polymer–enzyme inhibitor conjugates, in order to improve the protecting effect of the polymeric drug carrier matrix towards enzymatic degradation. A chitosan–Bowman Birk inhibitor and chitosan–elastatinal conjugate, were added to enteric coated delivery systems targeted to the small intestine. In contrast, the stomach-targeted calcitonin delivery system comprised a chitosan–pepstatin A conjugate to inhibit pepsinic degradation of the incorporated therapeutic peptide. The different calcitonin delivery systems were orally administered to rats and the plasma calcium level as a pharmacological response was determined. The oral application of calcitonin in ascorbic acid solution and control tablets comprising unmodified chitosan and, optionally, polymer–inhibitor conjugates, resulted in no significant effect. Calcitonin embedded in thiolated chitosan matrix tablets, however, led to a ≥ 5% decrease of the plasma calcium level. Thiolated chitosan tablets comprising reduced GSH displayed a significantly higher pharmacological efficacy compared with chitosan–TBA tablets lacking this permeation mediator. The strongest effect was achieved with the stomach-targeted system. The calcium level decreased by ≥ 10% and the effect held for ≥ 12 h [44].

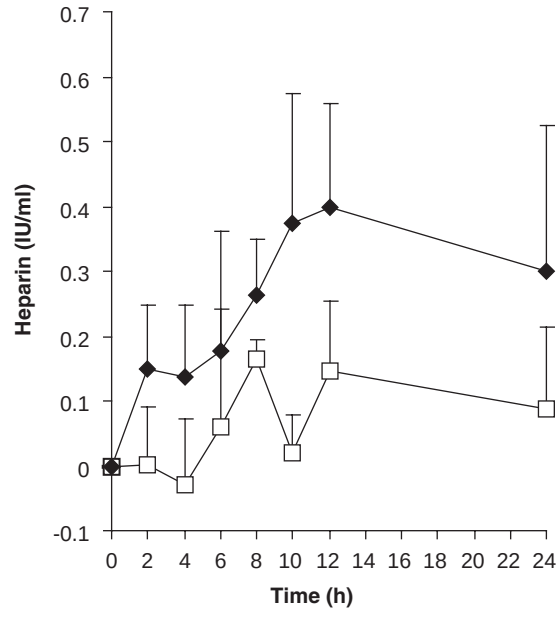


Figure 6. Comparison of the concentration profiles of heparin in plasma after peroral administration of heparin incorporated in tablets of polycarbophil–Cys and GSH (diamonds) and in tablets comprising unmodified polycarbophil (squares). Adapted from Kast *et al.* [25].
GSH:Glutathione.

5.3. Oral delivery of low-molecular-weight heparin

At present, low-molecular-weight heparin (LMWH) has to be administered via subcutaneous injections, which are often painful and occasionally dangerous, resulting in a poor compliance in self-administration. Accordingly, the oral administration of heparin would provide a higher therapeutic efficacy and

a greater ease for practitioners and patients. Due to its relatively large size and the negative charges, the GI absorption after peroral administration is very poor [19]. Several research groups have, therefore, sought suitable strategies to facilitate the GI absorption of orally delivered LMWH. These attempts are mainly based on the use of permeation enhancing systems, such as organic acids or bases, bile salts or liposomes [19-26]. Sodium *N*–[8–(2–hydroxybenzoyl)amino]caprylate is an amino acid molecule shown to facilitate the GI absorption of codelivered heparin *in vivo* [53]. Unfortunately, most of the attempts have failed because the permeation enhancers are absorbed much more rapidly from the GI tract than the drug itself [25]. The potential of thiomers for the oral administration of LMWH was, therefore, evaluated. *In vivo* studies with different oral heparin formulations showed no statistically significant effect on the drug. In contrast, a significant effect of orally administered heparin could be achieved by utilising thiolated PAA, as illustrated in Figure 6. An absolute bioavailability of 19.9 ± 9.3% was thereby achieved [25]. Control tablets of heparin and unmodified PAA showed a slight increase in the bioavailability determined to be 5.8 ± 1.4%. Moreover, the thiomer delivery system displayed a prolonged efficacy of heparin compared with other formulations, with a maximum of 0.4 ± 0.16 IU/ml being reached after 12 h and the efficacy maintained for at least an additional 12 h.

6. Thiomer safety

In contrast to low-molecular-mass permeation enhancers being absorbed from the GI tract in significant quantities, causing, in many cases, various systemic toxic side effects, thiomers are not taken up from the GI tract. Riley *et al.* [54], for example, demonstrated that PAA, displaying a molecular mass of 140 kDa, cannot be absorbed from the GI tract. Accordingly, the polycarbophil–Cys conjugate with a molecular mass > 3 mDa, will not be absorbed as well. Hence, systemic toxic side effects can be excluded. Because the mucus turnover in humans is 12 – 24 h, an accumulation of thiomers in the GI tract can also be excluded. Moreover, studies focusing on the cytotoxicity of thiolated polymers demonstrated no significant increase in cytotoxicity due to the immobilisation of thiol groups on various, well-established, polymeric excipients, such as chitosan [55]. Furthermore, clinical trials performed with thiolated PAA showed no irritation, even on the ocular surface, representing a very sensitive mucosal membrane [56].

7. Expert opinion

Due to the immobilisation of thiol groups on polymeric excipients, such as poly(acrylates) and chitosans, their mucoadhesive, enzyme inhibitory and permeation-enhancing properties are significantly improved. As the cohesive properties are also strongly improved, due to a crosslinking process via disulfide bond formation within the polymeric network, a mainly diffusion-controlled, sustained release of thiomer-embedded drugs can be guaranteed. In comparison with oral 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 hydrophilic macromolecular drugs being embedded in different types of thiomers. According to these results, thiomers seem to represent a promising new generation of multifunctional polymers for the oral delivery of hydrophilic macromolecular drugs.

From the intellectual property point of view, thiomers offer the advantage, on the one hand, to be broadly protected by various patents and, on the other hand, can be easily combined with various other off-patent delivery technologies, such as the additional use of certain low-molecular-mass permeation enhancers and/or additional enzyme inhibitors if needed. By the development of such combinations showing a synergistic, or at least an additive effect, additional intellectual property can be generated.

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