

Multifunctional Polymeric Excipients in Non-Invasive Delivery of Hydrophilic Macromolecular Drugs: The Thiomers-Technology

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Introduction

Non-invasive delivery of hydrophilic macromolecular drugs such as peptides, nucleic acids and polysaccharides is one of the major challenges in modern pharmaceutical technology. Most of these potent therapeutic agents have to be administered via parenteral routes, which are inconvenient because of pain, fear and the risks associated with this type of administration. 'Injectable-to-non-invasive-conversions' and in particular 'injectable-to-oral-conversions' are therefore in high demand. In order to provide sufficient high bioavailability for non-invasive delivery systems, however, various barriers including the diffusion barrier based on the mucus gel layer covering mucosal membranes, the enzymatic barrier represented by secreted- and membrane-bound peptidases and the absorption barrier have to be overcome. Strategies to overcome these barriers include the use of enzyme inhibitors (Bernkop-Schnürch, 1998), permeation enhancers and multifunctional polymers (Bernkop-Schnürch *et al.*, 2001). In the case of multifunctional polymers, enzyme inhibiting and permeation enhancing effects can only take place if close contact of the polymer with the mucosa is provided, ideally for the whole period of drug release and absorption. Multifunctional polymers should therefore also offer strong mucoadhesive features.

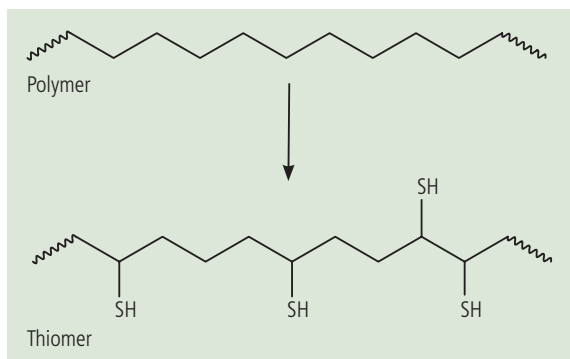


Figure 1 – Thiolated polymers-Thiomers.

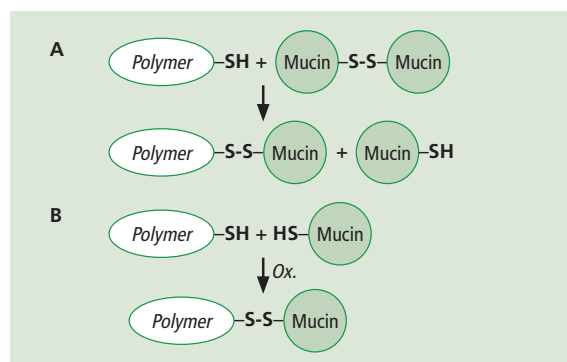


Figure 2 – Formation of covalent bonds between thiolated polymers and mucin glycoproteins. A: via thiol/disulfide exchange reaction; B: via an oxidation process (Adapted from Leitner *et al.*, 2003).

Among multifunctional polymers exhibiting all such properties, thiolated polymers (thiomers) are the most promising for non-invasive delivery of hydrophilic macromolecular drugs. Due to the immobilisation of thiol groups on well established multifunctional polymers such as poly(acrylates) and chitosans (see Figure 1), their mucoadhesive, controlled-release, permeation enhancing and enzyme inhibitory properties are greatly improved (Bernkop-Schnürch *et al.*, 1999).

Mucoadhesive Properties

Mucoadhesive drug delivery systems should provide close, prolonged contact of the drug with the mucosa, thus achieving a steep concentration gradient of the drug towards the absorption membrane; this is the driving force for passive drug uptake and subsequent higher bioavailability. Many theories have been proposed to describe mucoadhesion, and although the exact mechanism of adhesion of anionic polymers has not been thoroughly investigated, it is generally accepted that secondary molecular interactions with the mucus via van der Waal's forces and hydrogen bonding are primarily

Polymer	Degree of modification ($\mu\text{mol/g}$)	Adhesion time (h)	Improvement ratio
Polycarboxophil-cysteine	12	>10	2.1
Poly(acrylic acid)-cysteine	695	22	13
Chitosan-4-thiobutylamidine	682	>160	>94
Chitosan-thioethylamidine	140	24	8.9
Chitosan-thioglycolic acid	27	4	5

Table 1– Comparison of the mucoadhesive properties of various thiomers. Mucoadhesion studies were performed via rotating cylinder method.

Improvement ratio = adhesion time of thiomers/adhesion time of corresponding unmodified polymer.

responsible (Gu *et al.*, 1988). Cationic polymers adhere to the negatively charged mucus mainly due to electrostatic forces (Woodley, 2001). In thiomers, mucoadhesion occurs by the formation of additional covalent bonds between the thiol groups and the cysteine-rich subdomains of mucus glycoproteins as illustrated in Figure 2 (Leitner *et al.*, 2003). This significantly improves the mucoadhesive properties of thiomers. The results of some mucoadhesion studies are shown in Table 1.

Controlled Drug Release

Controlled drug release out of polymeric carrier systems is in many cases essential for an increased absorption rate and enhanced bioavailability. 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 poly(acrylic acid), for instance, were stable for more than 48 hours in simulated intestinal fluid without any observable erosion. The thiol functions of thiomers enable them to form inter- and intramolecular disulfide bonds. This cross-linking results in a highly stable polymeric network. Such thiomers have been demonstrated to provide an almost zero-order release kinetic for drugs such as insulin (Clausen and Bernkop-Schnürch, 2001). The reason for this sustained release is the cross-linking within the matrix

tablet, which guarantees a tightened three-dimensional polymeric network leading to a more controlled release. Thiol/disulfide exchange reactions between insulin and the thiolated polymer could thereby be excluded. Apart from a sustained release of hydrophilic macromolecular drugs over many hours, however, rapid drug release within minutes can also be guaranteed (Krauland and Bernkop-Schnürch, 2004).

Permeation Enhancing Properties

Strategies to overcome the absorption barrier are based on the co-administration of permeation enhancers. Various classes of small molecules have proved useful in improving permeation across intact epithelial membranes. Some of them, however, can cause mucosal damage or can even enter the systemic circulation, leading to systemic toxic effects. Thiomers display some advantages compared with small molecular enhancers such as their strong mucoadhesive properties, which allows them to remain concentrated at the area of drug absorption. Moreover, as high molecular mass polymers will not be absorbed from mucosal barriers, systemic side-effects can be excluded (Riley *et al.*, 2001). Some thiomers also show a strong permeation enhancing effect. The results of some permeation studies are listed in Table 2.

Enzyme Inhibitory Properties

In peptide drugs, enzymatic degradation on mucosal membranes can be regarded as one of the main reasons for the poor bioavailability of this type of hydrophilic macromolecular drug. Hence, drug delivery systems providing a protective effect towards secreted as well as membrane bound enzymes are in high demand. Two major strategies have been pursued: the addition of low molecular mass enzyme inhibitors and the use of polymers showing enzyme inhibitory properties. As low molecular mass enzyme inhibitors are extensively diluted in the GI tract, and in many cases absorbed faster than the peptide drug itself, their efficacy remains questionable. Furthermore, various side-effects as well as feedback regulations leading to an increased enzymatic activity cannot be avoided. Thiomers are promising candidates

Permeation Enhancer	Test Compound	Enhancement Ratio (P_{app} thiomers/ P_{app} unmodified control polymer)	Apparent Permeability Coefficient [$P_{app} \times 10^{-6}$ (cm/sec)]
Chitosan-4-thiobutylamidine	rhodamine	1.8	1.5 \pm 0.7
Chitosan-4-thiobutylamidine/GSH	rhodamine	3.6	3.0 \pm 1.2
Chitosan-cysteine	bac-FITC	signif.	n.a.
poly(acrylic acid)-homocysteine	Na-Flu	2.4	5.9 \pm 1.97
Polycarboxophil-cysteine	LMWH	1.1	0.19 \pm 0.04
Polycarboxophil-cysteine/GSH	LMWH	2.2	0.39 \pm 0.02
Polycarboxophil-cysteine/GSH	bac-FITC	2.06	9.94 \pm 0.82
Polycarboxophil-cysteine/GSH	Na-Flu	2.93	14.64 \pm 0.93
Polycarboxophil-cysteine	Na-Flu	1.57	5.27 \pm 0.11

Abbreviations:
bac-FITC: fluorescein-isothiocyanate labelled bacitracin; LMWH: low molecular weight heparin; Na-Flu: sodium fluorescein; rhodamine: rhodamine 123

Table 2 – Permeation enhancing properties of thiomers in comparison with the corresponding unmodified polymers tested on freshly excised intestinal mucosa of guinea pigs.

within the group of enzyme inhibiting polymers. The inhibitory properties of poly(acrylates) on intestinal proteases were first reported by Hutton *et al.* (1990).

Thiomer Delivery Systems

Dosage forms comprising thiomers are matrix tablets and particulate delivery systems. In matrix tablets, a higher stability of the delivery system can be achieved as thiolated polymers are capable of forming disulfide bonds within the polymeric network. This 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 (Guggi *et al.*, 2004) and also by adding a sugar alcohol such as mannitol to the tablet.

If polymers are used as drug carrier matrices for tablets, the polymer forms a gel after coming into contact with mucosal membrane liquids. In order to ensure swelling of orally administered tablets directly on the intestinal mucosa, tablets can be enterically coated (Marschütz *et al.*, 2000). In stomach-targeted delivery systems, coating tablets with triglycerides has been sufficient to provide swelling of the dosage form once it has reached the target. Particulate formulations display *per se* a prolonged residence time on mucosal membranes compared with single-unit dosage forms (Coupe *et al.*, 1991). This residence time on these membranes is even further improved when they exhibit mucoadhesive properties. The immobilisation of thiol groups on microparticles means that the mucoadhesive properties are improved. Poly(acrylic acid)-cysteine microparticles, for example, are almost 14 times more adhesive on the intestinal mucosa than unmodified polymer particles (Krauland and Bernkop-Schnürch, 2004). Thiomer microparticles can be prepared via various techniques such as the emulsification solvent evaporation technique or via jet milling. Due to the formation of disulfide bonds within the particles, they do not disintegrate under physiological conditions. In addition, controlled drug release can be achieved.

Oral Delivery of Calcitonin

Guggi *et al.* (2003) evaluated calcitonin tablets comprising thiolated chitosan. Small intestine targeting and stomach targeting formulations were generated. The delivery systems contained salmon calcitonin, permeation mediator reduced glutathione and some polymer-enzyme inhibitor conjugates in order to improve the protective effect of the carrier matrix to enzymatic degradation. The oral application of calcitonin in ascorbic acid solution and control tablets comprising unmodified chitosan resulted in no significant effect. Calcitonin embedded in thiolated chitosan matrix tablets led to a more than 5% decrease of the plasma calcium level. Thiolated chitosan tablets comprising reduced glutathione exhibited an even higher pharmacological efficacy compared with chitosan-4-thiobutylamidine tablets lacking this permeation mediator. The highest pharmacological efficacy was achieved with

the stomach targeted system where the calcium level decreased by over 10%.

Oral Delivery of Insulin

Marschütz *et al.* (2000) developed insulin tablets based on the thiolated polymer polycarboxiphilcysteine 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 was sustained for over 80 hours. In another study, Caliceti *et al.* (2004) formulated insulin tablets with poly(acrylic acid)-cysteine as drug carrier matrix. After oral administration to diabetic mice, these tablets led to a decrease of the blood glucose level of almost 60% and the effect lasted for 20 hours. The results of this study are shown in Figure 3.

Oral Delivery of Low Molecular Weight Heparin

Low molecular weight heparin is still administered via sc injections, which are often very painful. Accordingly, the oral administration of heparin would provide greater therapeutic efficacy and less discomfort for patients. Due to its relatively large size and the negative charges, gastrointestinal absorption after peroral administration is very poor. Attempts have been based on the use of permeation enhancing systems such as organic acids or bases, most of which have failed because the permeation enhancers are absorbed much more rapidly from the GI tract than the drug itself (Kast *et al.*, 2003). The potential of thiomers for oral administration of low molecular weight heparin has been evaluated. *In vivo* studies with different oral heparin formulations showed no statistically significant effect on the drug. A significant effect of orally administered heparin was achieved using thiolated poly(acrylic acid) as illustrated in Figure 4. An absolute bioavailability of $19.9 \pm 9.3\%$ was achieved. Control tablets of heparin and unmodified poly(acrylic acid) showed a slight increase in the bioavailability determined of $5.8 \pm 1.4\%$.

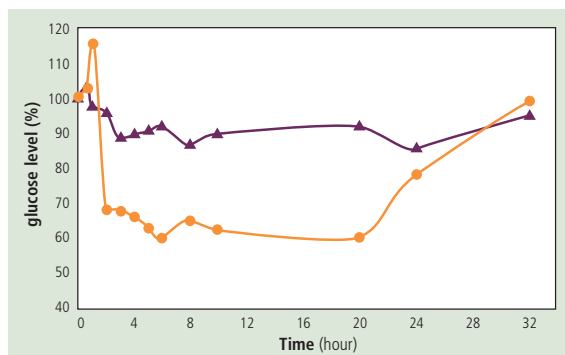


Figure 3 – Decrease in blood glucose level in diabetic mice after oral administration of pegylated insulin loaded tablets (●) and pegylated insulin in solution (▲). Each point represents the mean \pm SD of ten experiments. (Adapted from Caliceti *et al.*, 2004).

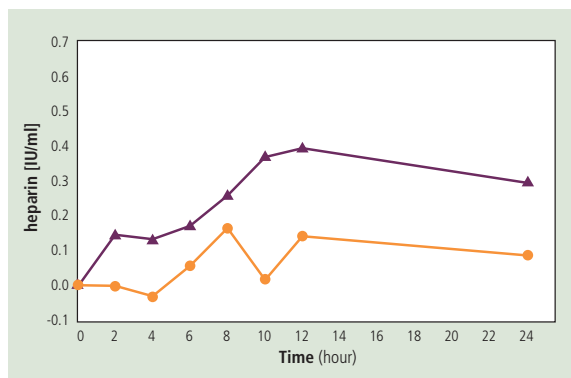


Figure 4 – Comparison of the concentration profiles of heparin in plasma after peroral administration of heparin incorporated in tablets of poly(acrylic acid)-cysteine and glutathione (▲) and in tablets comprising unmodified poly(acrylic acid) (●) (Adapted from Kast et al., 2003).

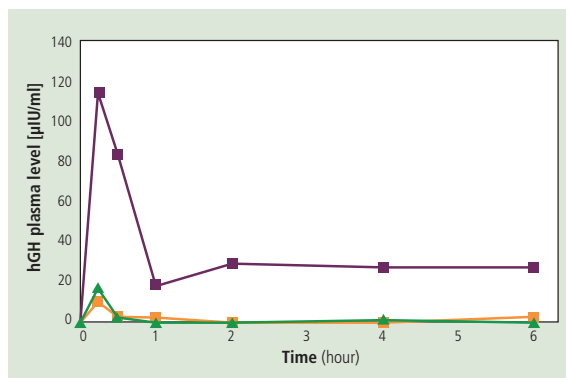


Figure 5 – Plasma concentration-time profiles following intranasal administration of hGH incorporated in Polycarbophil-cysteine/GSH (■), Polycarbophil-cysteine (□) and physiological saline (▲). Data represent the mean \pm S.D. of n=4-5 for all delivery systems (Adapted from Leitner et al., 2004).

Nasal Human Growth Hormone (hGH)

An aqueous nasal gel formulation was developed consisting of polycarbophil-cysteine, glutathione and hGH in final concentrations of 0.3%, 0.5% and 0.6% (m/v), respectively. Controls of 0.3% (m/v) polycarbophil-cysteine gel and physiological saline were prepared containing the same amount of hGH. The polycarbophil-cysteine/glutathione/hGH nasal gel delivery system led to a significantly higher hGH plasma concentration compared with both controls as shown in Figure 5. In addition, the thiomers gel delivery system was able to prolong the efficacy of hGH (Leitner et al., 2004).

Conclusion

Due to the immobilisation of thiol groups on polymeric excipients such as chitosans and poly(acrylates) their mucoadhesive, permeation enhancing and enzyme inhibitory properties are significantly improved. Compared with oral drug delivery systems comprising unthiolated polymers, the efficacy of delivery systems comprising thiolated polymers is significantly higher. Thiomers appear to represent a promising new generation of multifunctional polymers for non-invasive delivery of hydrophilic macromolecular drugs. Companies already making use of this novel technology are **MucoBiomer**, Leobendorf, Austria and **ThioMatrix**, Innsbruck, Austria.

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