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THIOLATED MUCOADHESIVE POLYMERS (THIOMERS)

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ABSTRACT

Mucus turns over, and anything resting on it is cleared within minutes to a few hours, which sets a hard ceiling on how long a conventional mucoadhesive dosage form can act at an absorption site. Thiolated polymers, or thiomers, were designed to break that ceiling by carrying free sulfhydryl groups that form covalent disulphide bonds with the cysteine-rich subdomains of mucus glycoproteins rather than relying on the weak secondary interactions of first-generation mucoadhesives. This review traces the class from the 1999 report of cysteine-grafted polycarbophil to the preactivated and S-protected derivatives and thiolated cyclodextrins of the present decade. The covalent anchoring mechanism is examined against the direct evidence for it, chiefly the loss of adhesion when

free cysteine competes for the polymer's thiols. Mucoadhesion gains reported across the literature span from roughly six-fold for thiolated hyaluronic acid to about 100-fold for chitosan-thiobutylamidine and close to 960-fold for a preactivated poly(acrylic acid) conjugate, though these numbers come from incompatible assays and are not directly comparable. Beyond adhesion, thiomers enhance permeation through glutathione-dependent inhibition of the protein tyrosine phosphatase that regulates tight junctions and of the P-glycoprotein efflux pump, and they prolong drug release by in situ disulphide crosslinking. In vivo work has shown oral absolute bioavailabilities near 20% for low-molecular-weight heparin and measurable systemic effect for salmon calcitonin and insulin in rodents. The one clear translational success is ocular: chitosan-N-acetylcysteine eye drops reached the market for dry eye disease and improved tear film thickness, break-up time and symptom scores in small clinical studies. Oral human evidence remains absent, which is the gap the field must now close.

Keywords: thiomers; thiolated polymers; mucoadhesion; disulphide bonds; permeation enhancement; chitosan; drug delivery; mucosa.



1. Introduction

A drug delivered to a mucosal surface competes against the clearance of that surface. The mucus blanket covering the gastrointestinal tract, the eye, the nasal cavity and the vagina is secreted continuously and shed continuously, so a dosage form that merely sits on it is carried away on the same schedule as the mucus itself. For the gastrointestinal tract that residence is on the order of minutes to a few hours, and for the precorneal tear film it is shorter still. Mucoadhesion was the pharmaceutical answer to this problem: make the dosage form stick, hold it against the absorbing epithelium, and both the duration of contact and the concentration gradient driving absorption improve ¹.

The first generation of mucoadhesive polymers, polyacrylates such as polycarbophil and carbomer, cellulose derivatives, alginate and chitosan, adhere through physical mechanisms. Their chains interpenetrate the mucus gel and are held there by hydrogen bonds, van der Waals forces and chain entanglement ². These interactions are real but weak and reversible, and they are undone by the normal hydration and turnover of the mucus layer. The adhesion they provide is measured in minutes, not hours, and it degrades further as the dosage form hydrates and its surface chains dilute into the overlying mucus.

The conceptual shift came in 1999, when Bernkop-Schnürch and colleagues attached cysteine to polycarbophil and reported that the modified polymer bound to intestinal mucosa far more tenaciously than the parent ³. The reasoning

was borrowed from the mucus gel itself. Mucin glycoproteins are held together partly by disulphide bonds between cysteine-rich domains, and the same chemistry that crosslinks the gel can anchor a polymer to it if the polymer carries thiol groups of its own. A thiol-bearing polymer, a thiomers, can form covalent disulphide bridges with mucin rather than relying on physical entanglement alone. That single idea generated a research programme that now spans three structural generations and a catalogue of base polymers, and it is the subject of this review. The focus is the chemistry that defines the class, the mechanistic evidence that the covalent bond actually forms and actually matters, the functional consequences for adhesion, permeation and release, and the extent to which any of it has reached a patient.

2. Chemistry and classification

2.1 Immobilising thiols on a polymer backbone

A thiomers is a hydrophilic polymer onto which sulfhydryl-bearing ligands have been covalently fixed, most often through amide or, less commonly, through ester bonds. The synthetic routes are chosen by which reactive handle the base polymer offers. Cationic polymers carrying primary amines, chitosan above all, are coupled to thiol-bearing carboxylic acids such as thioglycolic acid or cysteine using carbodiimide chemistry, which condenses the polymer amine with the ligand carboxyl to give an amide ⁴. Anionic polymers such as poly(acrylic acid) and polycarbophil, which present carboxyl groups, are coupled instead to the amine of cysteine or



cysteamine by the same carbodiimide activation³. A second route to cationic thiomers avoids a separate ligand entirely: 2-iminothiolane (Traut's reagent) reacts with a polymer amine in one step to install an amidine linkage terminating in a thiol, which is how chitosan-thiobutylamidine, usually abbreviated chitosan-TBA, is made⁵.

Reaction conditions matter as much as the choice of ligand. Carbodiimide coupling is run at mildly acidic pH, around 5 to 6, because this protonates the thiol enough to suppress its oxidation during the reaction while still allowing the carboxyl activation to proceed, and the product is then purified by dialysis and isolated by lyophilisation under conditions that keep the thiols reduced. A conjugate worked up carelessly arrives already partly oxidised, with interchain disulphides that reduce both its solubility and the thiol pool available to bind mucin.

The practical measure of a thiomers is the quantity of free thiol it carries, expressed as micromoles of sulfhydryl per gram of polymer and determined colourimetrically with Ellman's reagent, 5,5'-dithiobis(2-nitrobenzoic acid), which releases a yellow nitrothiobenzoate anion in stoichiometric proportion to the thiol it reacts with⁶. The complementary quantity, the disulphide already formed within the conjugate, is measured by reducing it and assaying the thiol released, so that the reduced and oxidised fractions together describe the state of the polymer before it is dosed. Reported thiol contents vary widely with the base polymer and the coupling conditions. The original chitosan-thioglycolic acid conjugates carried between 6.6

and 38.2 micromoles per gram⁴, whereas optimised chitosan-TBA reached 264 micromoles per gram⁷, and non-ionic thiolated cyclodextrins have been prepared with more than 1100 micromoles per gram⁸. Thiol content is not a free parameter to be maximised. Too few thiols and the polymer behaves like its unmodified parent; too many, and the conjugate oxidises and crosslinks before it is ever administered.

2.2 Three generations

The field is usefully divided into three generations by how the thiol is presented. First-generation thiomers carry the thiol in its reduced, free form: chitosan-thioglycolic acid, chitosan-cysteine, chitosan-TBA, thiolated poly(acrylic acid), polycarbophil-cysteine, and later thiolated alginate, hyaluronic acid, gelatin and others⁹. Their weakness is chemical. A free aliphatic thiol is only reactive toward disulphide formation when it is deprotonated to the thiolate, and at the mildly acidic to neutral pH of most mucosal surfaces a large fraction stays protonated and unreactive. Worse, the same thiols oxidise on storage, forming intramolecular and intermolecular disulphides that consume the groups meant to bind mucin¹⁰.

The second generation, preactivated thiomers, was the answer to the reactivity problem. Here the polymer thiol is capped before administration with an aromatic thiol leaving group, typically 2-mercaptanonicotinic acid or a pyridyl derivative, to form a defined mixed disulphide on the polymer. The capped thiol cannot oxidise, so the conjugate is stable, and it does not depend on deprotonation to react, because thiol-disulphide exchange with



mucin proceeds readily across a broad pH range. Iqbal and colleagues preactivated poly(acrylic acid)-cysteine with 2-mercaptosuccinic acid and reported adhesion and viscosity gains far beyond the unprotected polymer, with a rotating-cylinder residence time up to 960-fold that of unmodified poly(acrylic acid) for the highest-molecular-weight conjugate ¹¹. The closely related S-protected thiolated chitosans, in which a defined fraction of thiols is capped, combine storage stability with retained permeation-enhancing and efflux-inhibiting activity ^{12,13}.

The third development is not a backbone chemistry but a shift of host. Thiolated cyclodextrins carry thiol groups on a cyclic oligosaccharide that also forms inclusion complexes with poorly soluble drugs, so a single excipient provides mucoadhesion, permeation enhancement and solubilisation at once. Non-ionic thiolated beta-cyclodextrins raised the aqueous solubility of miconazole up to 257-fold while improving mucoadhesion roughly 46-fold over the native cyclodextrin ⁸.

3. The mucoadhesion mechanism and the evidence for it

3.1 Covalent bonding to mucin

The claim that distinguishes thiomers from every earlier mucoadhesive is that they bond covalently to mucus. The structural basis is specific. Secreted gel-forming mucins such as MUC2 in the gut and MUC5AC at the ocular surface are large glycoproteins whose protein cores carry cysteine-rich domains at their termini, and these cysteines form the disulphide bridges that

polymerise mucin monomers into the gel network ^{14,15}. Some of these cysteines are not fully oxidised and remain available. A thiomers thiol, once deprotonated or presented as a reactive mixed disulphide, undergoes thiol-disulphide exchange with these mucin cysteines, forming a new covalent R-S-S-mucin bond that ties the polymer into the gel. Figure 1 sets out the sequence, from contact and swelling through disulphide exchange to the downstream permeation and release effects.

The mechanism is attractive, but attractiveness is not evidence. The decisive experiment is a competition study reported by Leitner and colleagues, who measured the adhesion of thiolated poly(acrylic acid) tablets to porcine mucosa with and without free cysteine in the medium ¹⁶. Free cysteine, a small-molecule thiol, competes for the polymer's reactive groups and for the mucin cysteines, and if adhesion depends on disulphide exchange then adding it should weaken the bond. It did, significantly, which is difficult to explain by any purely physical model of adhesion and is the strongest single piece of evidence that the covalent bond forms and carries the load. Supporting this, the adhesion of thiomers tracks their thiol content across conjugate series, and it responds to the oxidation state of the polymer in the way a disulphide mechanism predicts.

3.2 How large is the effect, and how comparable are the numbers

The magnitude of the mucoadhesion gain is the headline result of most thiomers papers, and it is also the most frequently misread. The original



polycarbophil-cysteine raised the total work of adhesion to porcine intestinal mucosa from 104 microjoules for the unmodified polymer to 280 microjoules for the most substituted conjugate, a 2.7-fold gain measured by tensile testing ³. Chitosan-thioglycolic acid conjugates improved the same tensile metric 6.3- to 10.3-fold over unmodified chitosan, scaling with thiol content ⁴. Chitosan-TBA at high substitution reached roughly 100-fold by the same family of methods, and also stayed on a rotating cylinder about four times longer than a low-molecular-weight analogue ⁷. Thiolated hyaluronic acid prolonged mucosal residence about 6.5-fold and swelled some 3.5-fold more than the parent ¹⁷. At the extreme, the preactivated poly(acrylic acid) conjugate reported a 960-fold rotating-cylinder residence gain ¹¹.

These figures are not on a common scale, and treating them as a ranking is a mistake the secondary literature repeats. Total work of adhesion from a tensile test, residence time on a rotating cylinder, and the detachment force from a texture analyser measure different physical quantities under different hydration and contact conditions, and a 960-fold result from one assay cannot be set beside a 10-fold result from another as though the polymers had been compared head to head. Grabovac and colleagues made the point directly by testing a panel of polymers under one protocol and finding that measured mucoadhesion depends heavily on the method, the pH and the way the sample is prepared, with freeze-dried thiomers prepared at acidic pH performing best ¹⁸. The defensible summary is

qualitative and still striking: thiolation reliably multiplies mucoadhesion, with reported gains across the literature ranging from a few-fold to more than two orders of magnitude ^{2,19}, and the preactivated derivatives sit at the top of that range. Figure 2 places representative values from several conjugate classes on a single logarithmic axis, with the caveat that the underlying assays differ.

The sources of that disagreement are worth naming, because they explain why the numbers cannot be pooled. The tensile test pulls a hydrated polymer disc off a mucosal sample and reports the total work of adhesion as the area under the force-displacement curve, which captures the energy of detachment but depends strongly on the contact time and the applied load. The rotating-cylinder method mounts the sample on a cylinder turning in buffer and times how long the polymer stays attached, which rewards cohesive, slowly eroding gels and penalises ones that dissolve, so a highly crosslinking preactivated thiomers scores far higher here than on a tensile test. Rheological synergism, the excess viscosity of a polymer-mucin mixture over the sum of its parts, measures the molecular interaction directly but says nothing about the force needed to detach a dosage form. Each assay answers a different formulation question, and a polymer optimised against one can look ordinary against another. A review that reports fold-changes without naming the assay behind each one invites exactly the false comparison that the primary authors were careful to avoid.



4. Beyond adhesion: permeation enhancement and controlled release

4.1 Opening the paracellular route and inhibiting efflux

Prolonged contact is of limited use if the drug cannot cross the epithelium, and the most consequential discovery about thiomers is that the same thiol chemistry that anchors them also enhances permeation. The effect is indirect and runs through glutathione. Clausen and colleagues showed that thiolated polycarbophil reduces oxidised glutathione back to its active reduced form at the epithelial surface and that reduced glutathione is what drives the permeation effect²⁰. In guinea pig intestinal mucosa mounted in Ussing chambers, reduced glutathione alone raised the permeation of the paracellular marker sodium fluorescein about 1.9-fold, and the thiomers-glutathione system raised it 2.9-fold while lowering transepithelial electrical resistance by about 20%²⁰. The mechanistic target is a protein tyrosine phosphatase that controls the tight junction. Glutathione, maintained in its reduced state by the thiomers, inhibits this phosphatase, the junction proteins stay phosphorylated, and the paracellular space opens transiently to let hydrophilic molecules through. The distinction from a classical surfactant enhancer is worth drawing. A surfactant perturbs the membrane non-specifically and in proportion to dose carries a cytotoxic cost, whereas the thiomers-glutathione effect acts on a regulatory enzyme and is self-limiting, since it depends on the polymer being present and reverses as the polymer is cleared.

That is the difference between prising the barrier open and persuading it to open itself.

The same reducing environment acts on efflux. The P-glycoprotein and multidrug-resistance-associated protein pumps that eject many drugs back across the apical membrane are sensitive to the local thiol-disulphide balance, and thiomers-glutathione systems inhibit them. Föger and colleagues embedded rhodamine-123, a model P-glycoprotein substrate, in thiolated chitosan minitables and measured a 3.0-fold increase in its oral bioavailability in rats compared with the unmodified carrier²¹. S-protected thiolated chitosan showed both the permeation-enhancing and the efflux-inhibitory activity in combination, which is the property that makes it attractive for orally delivered substrates of these pumps¹². The permeation effect is transient and reverses as the polymer is cleared, which is the behaviour wanted from an absorption enhancer: open the barrier while the drug is present, then let it close.

4.2 Controlled release through *in situ* crosslinking

A thiomers that can form disulphides with mucin can also form them with itself. Once hydrated, free thiols on neighbouring chains oxidise to interchain disulphide bonds, and the dosage form crosslinks in place into a more rigid, more cohesive gel. This stiffening raises the viscosity of the hydrated matrix, slows the diffusion of entrapped drug and slows erosion, so a thiomers matrix sustains release without any separate rate-controlling membrane⁹. The same *in situ* gelation stabilises the dosage form against the shear and dilution it meets at the mucosal



surface, which is part of why thiomers tablets hold their position and their drug load over hours rather than minutes.

4.3 Stability, which the preactivation step was designed to protect

The reactivity that makes first-generation thiomers useful also makes them fragile. Free thiols oxidise on storage, and an oxidised thiomers has spent the groups it needed to bind mucin and enhance permeation. A six-month study of polycarbophil-cysteine and chitosan-thioglycolic acid found that as powders the conjugates lost free thiol only under the harshest condition tested, 20 degrees Celsius at 70% relative humidity, while compressed into tablets both stayed stable across all storage conditions, which points to dry, compacted, low-humidity formulation as the practical safeguard¹⁰. Preactivation addresses the problem at its source by capping the thiol so it cannot oxidise, and the stability of S-protected conjugates is one of their principal advantages over the first generation^{11,13}.

5. Base polymers and the breadth of the platform

Chitosan is the most studied thiomers backbone, for reasons that go beyond availability. It is cationic, which gives it a baseline electrostatic attraction to the anionic sialic acid and sulphate groups of mucin, and thiolation adds the covalent bond on top of that attraction²². Thiolated chitosans improve mucoadhesion several-fold to roughly 100-fold depending on substitution and method, improve paracellular permeation of

hydrophilic markers, and have been formulated as tablets, microparticles, nanoparticles and liposome coatings²². The anionic thiomers, thiolated poly(acrylic acid) and polycarbophil, are the other mainstay and are preferred where a cationic charge is unwanted or where the preactivated high-performance derivatives are required^{3,11}.

The platform now reaches well beyond these two. Thiolated hyaluronic acid brings the biocompatibility and the receptor affinity of hyaluronan to the class¹⁷. Thiolated alginate exploits a polymer already valued for mild ionotropic gelation²³. Thiolated cyclodextrins fold solubilisation into the same molecule as adhesion⁸. Thiolation has also been applied to gelatin, pectin, carrageenan and cellulose derivatives, and the common finding across all of them is the same: grafting thiols onto a mucoadhesive polymer multiplies its adhesion and, where tested, its permeation-enhancing capacity^{9,24}. The generality is itself an argument that the disulphide mechanism, not some peculiarity of one backbone, is what is being exploited.

6. In vivo and clinical evidence

6.1 Oral delivery of peptides and macromolecules in animals

The purpose of the chemistry is systemic or local drug delivery, and the animal evidence for oral peptide delivery is the part of the thiomers literature that most nearly delivers on the original promise. The clearest result is for low-molecular-weight heparin. Kast and colleagues formulated



it in thiolated polycarbophil with a glutathione system in enteric-coated tablets and measured an absolute oral bioavailability of 19.9% in rats, with therapeutic anti-factor-Xa activity sustained for 24 hours after a single dose²⁵. For a molecule whose oral bioavailability is otherwise negligible, roughly 20% is a large effect. Salmon calcitonin delivered orally in a thiolated chitosan matrix with a glutathione and enzyme-inhibitor system produced a significant and sustained fall in plasma calcium in rats, on the order of 10% maintained for at least 10 hours, where the unmodified-chitosan control was inactive²⁶. Thiolated chitosan tablets have also delivered insulin orally to non-diabetic rats with a measurable hypoglycaemic response²⁷. A later refinement coated liposomes with S-protected thiolated chitosan and raised the oral bioavailability of salmon calcitonin about 8-fold over the free peptide in rats, illustrating how the preactivated chemistry and a particulate carrier can be combined²⁸.

6.2 Nasal, buccal and other mucosae

The same approach has been taken to other mucosae with animal and ex vivo models. A thiolated chitosan system improved the nasal absorption of insulin in rats relative to unmodified chitosan²⁹. For the buccal route, thiolated chitosan combined with reduced glutathione raised the permeation of the peptide PACAP across excised porcine buccal mucosa about 78-fold over baseline, far exceeding a conventional surfactant enhancer tested alongside it, though this was an ex vivo permeation measurement rather than a systemic

one³⁰. These studies establish the breadth of the platform across mucosal sites while also showing how much of the evidence remains preclinical or ex vivo.

6.3 The ocular route, where thiomers reached patients

The one setting in which thiomers have clearly reached humans is the eye. The precorneal residence of a conventional eye drop is extremely short, which makes covalent anchoring to the ocular mucins especially valuable. An early ocular insert based on thiolated poly(acrylic acid) was evaluated in human volunteers and maintained a sodium fluorescein level in the tear film and cornea for several hours³¹. The decisive translation, however, came from chitosan-N-acetylcysteine, a thiolated chitosan marketed as an eye drop for dry eye disease. In a controlled randomised double-blind study, a single instillation raised optical-coherence-tomography tear film thickness from 3.9 to 4.8 micrometres and kept it elevated for 24 hours, significantly above placebo, and corneal surface damage improved in more than 60% of patients³². Two subsequent clinical case series supported the finding: in one, a daily drop for five days improved mean ocular surface disease index scores by about 18 points³³, and in another, tear break-up time rose from 4.1 to 6.9 seconds and Schirmer scores from 6.4 to 10.8 millimetres over one month, with the fraction of intact corneas rising from 12% to 64%³⁴. More recently, a thiolated hydroxypropyl-beta-cyclodextrin prolonged corneal residence in rabbits from about 6 minutes



to 24 minutes and raised aqueous-humour exposure of a model drug nearly 12-fold, extending the ocular rationale to the cyclodextrin generation ³⁵.

7. Safety, limitations and future directions

The biocompatibility data are reassuring as far as they go. The base polymers are established pharmaceutical excipients, the disulphide chemistry mirrors a reaction that occurs physiologically in the mucus itself, and in vitro cytotoxicity testing of thiolated and unmodified chitosan nanoparticles found the thiolation did not confer meaningful additional toxicity ³⁶. The marketed ocular product has accumulated real-world use without a safety signal that has halted it ³². None of this substitutes for the systematic chronic-toxicity and repeat-dose immunogenicity studies that an oral or systemic product would require, and those studies are largely absent from the public record. Two concerns specific to the chemistry deserve dedicated study. A permeation enhancer that opens the paracellular route also, in principle, admits luminal antigens and microbial products that the junction normally excludes, so the safety of repeated tight-junction modulation over months is a question the acute rodent studies cannot answer. The preactivating leaving groups raise a second question: 2-mercaptanonicotinic acid and pyridyl thiols are released stoichiometrically when the mixed disulphide reacts with mucin, and the systemic fate of that released small molecule, trivial for a single topical dose, is not trivial for chronic oral use and has not been characterised in the published literature.

The honest assessment of the field is that its chemistry is mature and its clinical translation is narrow. The mechanism is established, the mucoadhesion gains are real if inconsistently measured, the permeation and release effects are mechanistically coherent, and the animal pharmacokinetic results for oral peptides are genuinely large. Yet after more than two decades the only unambiguous human success is a topical ocular product, and there is no published human evidence for the oral delivery that motivated the class. Three problems explain the gap and define the work ahead. First, the mucoadhesion literature needs standardised, head-to-head assays so that the fold-change numbers mean something comparable; the present situation, where a 960-fold result and a 6-fold result come from unrelated methods, is not a basis for rational formulation choice. Second, the stability and manufacturability of first-generation thiomers are marginal, and the field should consolidate around the preactivated and S-protected chemistries that solved the oxidation problem ^{11,13}. Third, and most importantly, the oral peptide results that look so promising in rats have never been carried into a human trial, and until that step is taken the central claim of the field remains unproven in people. The ocular success shows the chemistry can be translated. The task now is to translate it where the need, and the original ambition, was greatest.

8. Conclusion

Thiomers took one correct idea, that a polymer carrying thiols can bond covalently to the cysteine-rich domains of mucin, and built from it



a versatile platform that outperforms physical mucoadhesives in adhesion strength, prolongs drug release through in situ disulphide crosslinking, and enhances epithelial permeation through glutathione-dependent inhibition of tight-junction phosphatase and efflux pumps. The preactivated and S-protected generations resolved the instability that limited the first conjugates, and the extension to cyclodextrins added solubilisation to the same molecule. The

evidence that the mechanism works is strong in vitro and in animals, and it has translated cleanly into a marketed ocular product for dry eye disease. The decisive test the field has not yet met is a human trial of oral thiomers peptide delivery, and that trial, built on the preactivated chemistry and a standardised measure of mucoadhesion, is the single most important next step.

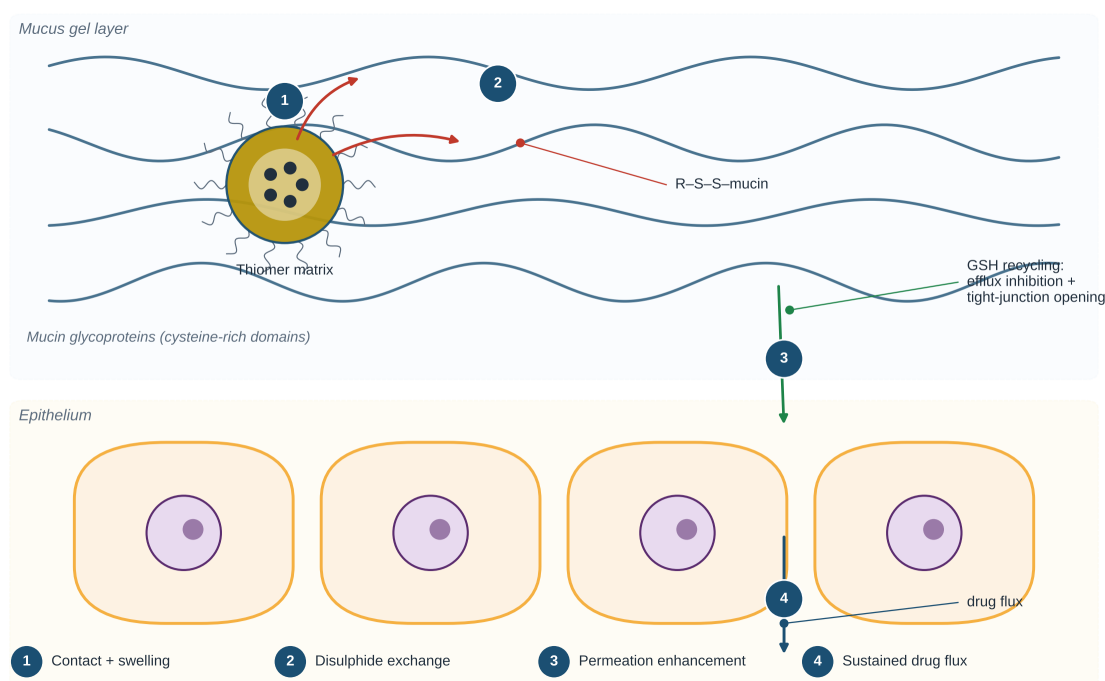


Figure 1. Mechanism of thiomers action at a mucosal surface. A hydrated thiomers matrix in the mucus gel layer (1) swells on contact, then (2) its free or preactivated thiol groups undergo thiol-disulphide exchange with the cysteine-rich domains of mucin glycoproteins, forming covalent R-S-S-mucin bonds that anchor the dosage form. (3) Thiomer-maintained reduced glutathione inhibits the tight-junction-regulating phosphatase and apical efflux pumps, enhancing permeation, so that (4) released drug crosses the epithelium at a sustained rate.

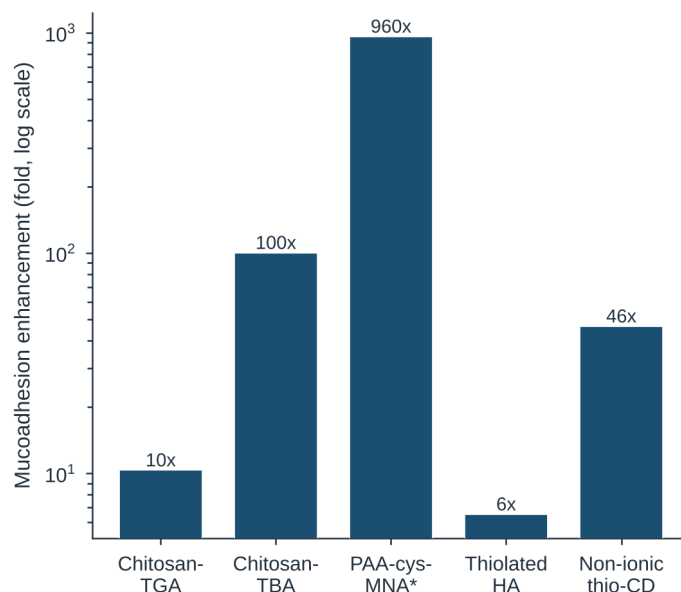


Figure 2. Representative mucoadhesion enhancement for five thiomers classes, expressed as fold increase over the corresponding unmodified polymer on a logarithmic axis: chitosan-thioglycolic acid (chitosan-TGA) ⁴, chitosan-thiobutylamidine (chitosan-TBA) ⁷, preactivated poly(acrylic acid)-cysteine-mercaptopnicotinic acid (PAA-cys-MNA) ¹¹, thiolated hyaluronic acid ¹⁷, and non-ionic thiolated cyclodextrin (thio-CD) ⁸.

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