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Review Article

Thiolated polymers: As bio inspired polymers

Jinendra Kr. Kochar Jain[1]*, Anupam Mishra[2], Anjali Basnett[1], Arnab Bagchi[1], Sudip Das[1], Sanjib Bahadur[1]

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Abstract
Thiomers are polymers that have had their backbones changed by adding thiol moieties by substitution reactions or direct oxidation reactions which provides them mucoadhesive characteristics and other additional qualities. Thiomers can be complexed with drugs that are less soluble and permeable to boost their absorption via the mucosal membranes by increasing contact time and prolonging their stay in the body due to mucoadhesion. Therefore, immobilisation of the thiol group improves the modified polymer's mucoadhesive characteristics by 2-140 folds. Different methods are used to characterise and stabilise the produced thiomers. Additionally, thiomers provide the body with a regulated release of the active medicinal substances. Chitosan, polyacrylic acid, sodium alginate, sodium carboxy methyl cellulose, guar gum, and other polymers can all be altered by thiolation. Drugs having low therapeutic compatibility are difficult to deliver with thiomeric formulations. Thiomer-containing micro- and nanopreparations can be made using a variety of methods, including covalent crosslinking, in situ gelation, radical emulsion polymerization, and emulsification. Thiomers are a promising pharmaceutical excipient with a wide range of applications nowadays in the testing phase of pharmaceutical technology.

INTRODUCTION

The word “Polymer” is derived from Greek roots “Poly” meaning many and “Meros” meaning parts. Polymers are long chain organic molecules assembled from many smaller molecules called as monomers. The word “polymer” means “many parts.” A polymer is a large molecule that is made up of many small repeating units. Polymers are considered to be a subset of macromolecules. A monomer is a small molecule that combines with other molecules of the same or different types to form a polymer. Polymers symbolize a vital component of pharmaceutical dosage forms. The formulation and clinical performance of pharmaceutical dosage forms, for example, solid dosage forms, implants, disperse systems, transdermal patches, and particulate systems, is reliant on the physicochemical properties of the polymers used in the formulation (1).

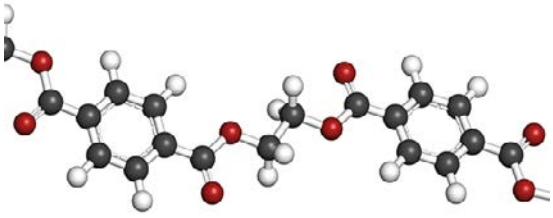

Figure 1: Basic structure of polymer (2)

IDEAL CHARACTERISTICS (3)

- It should be compatible with the biological environment, that is non-toxic and non-antigenic.
- It should be easy to administer.
- It should be biodegradable in nature or eliminated from the body after its function.
- It should be economical and easy to fabricate.
- It should provide drug attachment and release sites for drug-polymer linkages.

CLASSIFICATION (4)

Based on origin

- Natural Polymer: Polymers that occur in nature are called natural polymers, also called biopolymers. Example: Carbohydrates–starch, cellulose, glycogen. DNA, RNA. Proteins–Co llagen, Keratin, Albumin,
- Synthetic Polymers: The polymer that is synthesized in the laboratory is known as a synthetic polymer. Also known as artificial polymers. Example: Polyesters, polyanhydrides and polyamides.
- Semi-synthetic polymer: These are natural chemically modified polymers. Example: hydrogenated rubber, natural rubber, cellulose, cellulose nitrate, methyl cellulose, etc.

[1]*Department of Pharmaceutics, Himalayan Pharmacy Institute, Majhitar, East Sikkim, India 737136*
[2]*Packaging Department, Cipla Unit-II, Rorathang, Sikkim 737133, India*
For correspondence: jinendrajain123456@gmail.com

Based on Bio-stability

- Bio-degradable Polymer: In response to a chemical process like hydrolysis, these polymers progressively vanish from the administration site. Examples: proteins, carbohydrates, polyesters, etc.
- Non-biodegradable polymers: These are inert substances that are entirely removed from the application location. Example: ethyl cellulose, HPMC, acrylic polymers, silicones.

Based on Reaction mode of Polymerization

- Addition Polymers: • Olefin, diolefin, vinyl, and similar monomers are used to produce them. These polymers are created by a chain process that adds monomeric molecules to one another in fast succession.
  Examples: polyethylene, polypropylene, polystyrene.
- Condensation Polymers: Intermolecular reaction between the bifunctional and the multifunctional monomeric molecules with reactive functional groups such as -OH, -COOH, -NH2, -NCO, and others forms this compound.

Based on Interaction with Water

- Hydrogels: They swell but do not dissolve when in contact with water. Example: polyvinylpyrrolidone.
- Soluble Polymers: These are moderate molecular weight uncross-linked polymers that dissolve in water. Example: HPMC, PEG.

THIOLATED POLYMERS

Thiolated Polymers or Thiomers are modified in such a way for their mucoadhesive properties and other additive properties by adding thiol moieties in the backbone of the unmodified polymers by the process of substitution reactions or simple oxidation reactions. Drugs with less solubility and permeability can be complexed with thiomers to increase the absorptivity by the help of mucosal membranes by increasing the contact and prolong the stay in body due to mucoadhesion. The mucoadhesive properties of the modified polymer is increased by 2–140-folds by immobilizing the thiol group. The prepared thiomers are characterized and made stable by different techniques. Thiomers are also useful in controlled drug delivery system. The different polymers that are modified by thiolation are chitosan, polyacrylic acid, sodium alginate, sodium carboxy methyl cellulose, guar gum, etc. Thiomeric formulations deliver drugs with low therapeutic compatibility that is hard to achieve. Micro and nano thiomeric preparations are prepared by different processes such as covalent crosslinking, in-situ gelation, radical emulsion polymerization, and emulsification (5).

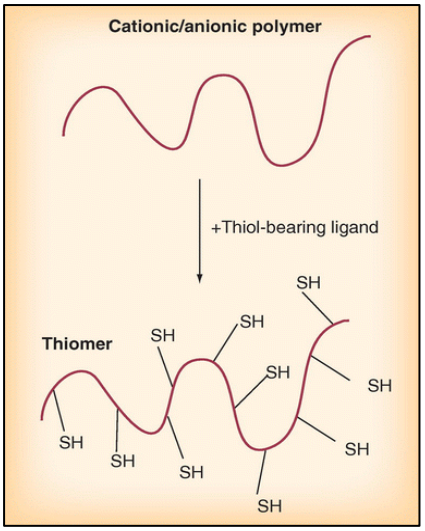

Figure 2: Thiolated Polymer (Thiomer) (6)

CHEMISTRY OF THIOMERS

The size and number of the polymer backbone, as well as the sulfhydryl groups, have a significant impact on the performance of thiomers. The capacity to generate disulfide bonds is influenced by several factors, including chain length, chain flexibility, and charged substructures of the polymer backbone. Thiomers are less reactive in polymers with longer chain lengths and less flexibility in the backbone (7). Molecular movements of high molecular mass thiomers of low chain flexibility are comparatively slow, thiols need time to get close to target thiol or disulfide to react. Steric hindrance is more effective. The possibility of thiols getting near to each other and forming crosslinks is decreased by the repulsion of polymer chains caused by the same charge (8). Polymer backbones are either biodegradable such as polysaccharides and proteins or non-biodegradable such as poly(meth)acrylates or silicones. Biodegradable thiomers are from the toxicological point of view preferred over non-biodegradable thiomers. For drug registration not only pharmaceutical excipient or medical device in biodegradable thiomers but toxicological studies of thiomer along with different degradation products must be shown. Thiomers' reactivity is also influenced by the degree of thiolation and the kind of sulfhydryl ligand (9).

The sulfhydryl ligands' pKa value significantly affects how reactive thiomers are since the thiolate anion, not the thiol group, is the reactive form. At physiological pH, the concentration of thiolate anions increases with decreasing pKa values of thiol groups (10). As thiolate anion is a greater nucleophile than thiol, the ratio of thiol or disulfide exchange process is inversely dependent on the pKa of attacking sulfhydryl ligands. Due to linear free energy relationship nucleophilicity of the thiolate anion with pKa causing opposite effect on the overall reaction. Thioamides

convert to thiols such as 2-mercaptopyridine or 2-mercaptonicotinic acid due to tautomerism which are highly reactive than simple alkyl. The Henderson Hasselbach equation is used to determine the thiolate anion concentration at a certain pH. A physiological pH of 7.2 is believed to be extremely significant, as shown by the ratio of thiolate anions to thiols of sulfhydryl ligands. Thiolate anion concentration at a certain ph is calculated using the Henderson Hasselbach equation. Physiological pH of 7.2 is likely to have high relevance, the ratio of thiolate anions to thiols of sulfhydryl ligands is shown (13). The covalent attachment of sulfhydryl ligands to some groups results in change of their chemical structure due to conjugation and altered pKa values. Carboxylic acid groups in polyacrylates i.e., a negative charged group raises the pKa value by destabilizing thiolate anions. In contrast amines in case of polyallylamine lowers the pKa by stabilizing thiolate thiolate anions. Thiolate anions are stabilised, reducing the pKa, thanks to hydrogen bond donors in the thiomer's immediate surroundings (11). The pKa of thiolated polymers is determined using either spectrophotometric titration or isothermal titration calorimetry. By immobilising sulfhydryl ligands and replacing the hydroxyl moieties on polymers with thiol groups, polymers can be thiolated. Thiomers are created by the copolymerization of several thiolated monomers (12).

METHODS OF THIOLATION

Thiolation by Traut's Reagent (13)

Traut's reagent, also known as 2-iminothiolane in chemistry, is a cyclic molecule frequently employed in thiolation processes. Traut's reagent reacts with primary amines to introduce a sulfhydryl group while retaining all charge properties. Initiating cross-linking or immobilisation reactions can then be done using these. The literature states that, depending on the protein size and concentration of the biopolymer, Traut's reagent and EDTA were added in variable quantities after the biopolymer was dissolved in a non-amine buffer (pH 7.4). The reaction mixture was then incubated for a further hour at room temperature. The unreacted reagent was tested for the presence of sulfhydryl groups using Ellman's reagent.

Thiolation by Dithiothreitol (DTT) Reduction (13)(14)

DTT is the substance 2,3-dihydroxy-1,4-dithiolbutane's trans isomer. It may decrease all biological sulfhydryl groups while maintaining free thiol groups even in the presence of oxygen. The biopolymer was dissolved in a pH 7.4 phosphate buffer. The amount and concentration of the biopolymer dictate the amount and concentration of DTT and EDTA, respectively. The reaction took place at room temperature for an hour. Sulfhydryl groups in an unreacted reagent were examined using Ellman's reagent.

Thiolation by Dithiolaromatic (PEG6-CONHNH$_2$) (15)

Dithiol aromatic is a substance that increases and relocates cysteine residues, forming non-native disulfide bonds as a consequence. Sodium hydrogen phosphate, sodium periodate, and the biopolymer were combined, and the experiment was run for 30 minutes in the dark. To the above-mentioned solution, phosphate buffer solution, dithiol aromatic, and ethanol mixtures were added. The reaction took place at room temperature for an hour. The unreacted reagent was then filtered, and Ellman's technique was used to identify the presence of sulfhydryl groups on the thiolated biopolymer.

Thiolation by Thiol Polyethylene Glycolamine (SH-PEG-NH$_2$) (13)(15)

The biopolymer was combined with EDAC or 1-thyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, phosphate buffer pH 7.4, and the mixture was incubated for about 2 hours on a rotator. Unreacted substances were removed, and sulfhydryl groups (-SH) on thiolated biopolymers were found using Ellman's reagent.

By Thioglycolic Acid Thiolation (13)(16)

Ethanolic biopolymer extract was dissolved in thioglycolic acid (TGA), and EDAC or 1-thyl-3-(3-dimethylaminopropyl) carbodiimide-hydrochloride was slowly added to the prepared mixture with constant stirring for about 3 hours. The unreacted compound was filtered, and the sulfhydryl groups were determined using Ellman's reagent (-SH)

CLASSIFICATION

Cationic Thiomer (17)

These are chitosan-based thiomers that are made by immobilizing a thiol group on the 2-amino position of the glucosamine in the polymer chain. Examples: chitosan cysteine, chitosan-thioglycolic acid, chitosan-thio butyl amidine.

Anionic Thiomer (18)

These thiomers' anionic targets are carboxylic acid groups. It is advantageous because sulfhydryl moieties may quickly form amide linkages with the cysteine and homocysteine ligands. These bonds are created by carboamides. Examples: polyacrylic acid-cysteamine, CMC-cysteine, and alginate-cysteine.

FEATURES

Bioadhesion

Mucoadhesion

Mucosal surfaces are an important application location for local therapy and non-invasive systemic medication delivery because they are easily accessible. Weak non-covalent linkages between polymers and mucins, like

hydrogen bonds, van der Waal forces, ionic, and hydrophobic interactions, are what allow for mucoadhesion. (19) Thiomers and their S-protected counterparts establish stronger sticky connections through thiol/disulfide exchange processes or thiol oxidation because of their ability to form disulfide bonds with cysteine substructures of the mucus gel layer. (20) In addition, the formation of disulfide connections between thiomer chains may be controlled and time dependent. This stable crosslinking results in a high cohesive polymer network and low adhesive bond failures. Furthermore, interpenetration improves sticky qualities. Stabilizing disulfide bonds are formed as the polymer penetrates the mucus gel layer. Likewise, thiomer is efficiently positioned within the mucus mesh work (7) Mucin is a high-molecular-weight, strongly glycolated protein found throughout the mucus membrane and contains cysteine-rich domains. Thiol interacts with these cysteines and creates disulfide bonds either by the oxidation of the thiol groups or through a thiol/disulfide exchange mechanism. The pH of the mucosa and the pKa of the thiol group both affect how much mucoadhesion occurs. The degree of mucoadhesion is influenced by the pKa of the thiol group, the pH of thiolated chitosan, and the pH of the surrounding medium. As a result, the ionic strength and pH state have no effect on the formation of disulfide bonds. (5)

Adhesion of keratinous surfaces

In the human body, epithelial cells and tissues including the stratum corneum, hair, and nails depend on keratins for mechanical stability and integrity. It contains a lot of cysteine, between 7 and 20 percent. Thiomers find it more enticing as a target as a consequence than mucin glycoproteins. It is possible to predict interactions between thiolated polymers and keratin similar to those reported in mucoadhesion based on thiol/disulfide-exchange reactions. (21) According to investigations, the adhesive properties of polycarbophil-cysteine on swine belly skin may be assessed with the aim of creating an efficient transdermal progesterone delivery method. (5) When compared to unmodified control polymers, the skin binding agent characteristics of hydrophilic and lipophilic thiol-modified polymer gels are significantly improved, with increases in adhesive forces of 6 times, 25 times, 9 times, and 5 times for the polymer gels of (acrylic acid)-cysteine, Carbopol 971-cysteine, carboxymethyl cellulose-cysteamine, thioglycolic acid, and silicone oil, respectively. (22) Additionally, thiolated polymers have demonstrated that they may be used in brand-new ways, notably in the textile sector. It was discovered that thiolated chitosan adhered to sheep wool keratin with a 2-fold higher degree than unmodified chitosan. (23)

Adhesion to membrane-associated proteins

Cell membrane proteins having cysteine-rich subdomains on the cell surface include integrins, scavenger receptors, epidermal development factor receptors, and insulin-like growth factor receptors. (24) The purpose of these cysteine-rich domains was to promote adhesion. Integrins are in charge of attaching cells into the matrix of cells and physically anchoring them there via the cell adhesion process (ECM). (25) Thiomers are drawn to cysteine-rich regions of membrane proteins. Various enzymes, such as protein tyrosine phosphatase, also have a thiol substructure, making them prime targets. Exofacial thiols are involved in several processes, such as cell absorption. Exofacial thiols are also involved in tight junction opening and efflux pump inhibition. (26) Exofacial thiols are found in larger concentrations in certain cancer cells, suggesting that this functional group could be a suitable site for targeted drug administration. (27) Monothiols can only create one disulfide bond with membrane proteins, but thiomers can form one or more disulfide bonds with membrane proteins, making them more strongly bound. Most of the research reveal the binding and interaction of thiomers with exofacial thiols, but it should be noted that the majority of these experiments and studies are based on immortalized cell lines or rodents. More recent investigations have found evidence of thiomers' improved affiliation with different human blood cells, providing more reliable data on their ability to stick to membrane-associated proteins. (28) For regulated cell adhesion and dissociation, a stimuli-responsive system made of thiolated chitosan and chondroitin sulphate was also developed. Because intrinsic disulfide bonds are formed and broken during oxidation and reduction, surface characteristics of the system were controlled towards human fibroblast adhesion in a reversible manner. The process might be used to biological applications such implantable medical devices as responsive and obedient surfaces. (29)

In-situ Gelation

Chitosan has the ability of crosslinking adds more to its mucoadhesive capability. After forming surface interaction, chitosan starts to form bonds with itself leading to even more strong adhesion. The thiolated chitosan sol gel transition occurs after 2 hours at pH 5.5. When the gelling property of thiolated chitosan is examined rheologically, it shows a considerable decrease in the thiol group concentration, indicating the production of disulfide bonds. In addition, thiolated chitosan has a higher elasticity than unthiolated chitosan. Thiolated polymers can result in a noticeably more pronounced increase in viscosity after application due to the strong crosslinking process brought on by the creation of disulfide bonds between the polymer

chains brought on by oxidation. For instance, thiolated chitosan showed a 10,000-fold increase in viscosity in a matter of minutes. Parenteral formulations, coatings, and food additives are just a few examples of alternative uses for these potent in situ gelling properties. (30)

Suppression of the efflux pump

Reversible efflux pump inhibition is a property of thiomers. This ability of numerous efflux pump substrates, such as anticancer treatments, antimycotic medications, and pain relievers, to be taken up by the mucosa, might considerably help to increase. (31) The mechanism of efflux pump inhibition (MRPs) involves the interaction of thiolated polymers with the channel-forming transmembrane domain of various efflux pumps, such as P-gp and multidrug resistance proteins. A pathway for delivering substrates outside of the cell is provided by P-gp, which comprises 12 transmembrane domains. A cysteine subunit may be found at positions 137 and 956 in two of these transmembrane domains, 2 and 11, respectively. Thiomers seem to enter the P-gp channel and form one or two disulfide linkages with either one or both the channel's cysteine subunits. This covalent bond may block the transporter's allosteric shift, which is necessary to transport medications outside of the cell. (32)

Permeation enhancement

The mechanism of the reversible tight junction opening by thiomers appears to be reliant on the inhibition of protein tyrosine phosphatase, which is involved in the tight junction closure process. (33) As a result, their permeation-enhancing impact can be sustained for a longer period of time, and the auxiliary agent's systemic harmful side effects can be avoided. (34)

Controlled drug release

Sustained drug release allows for the maintenance of an extended therapeutic level of drugs having a short elimination half-life. This can lead to greater compliance by lowering the frequency of doses. The release of medications from polymeric carrier systems may be managed using a straightforward diffusion approach. The polymeric network's rapid degradation and/or disintegration, however, have thus far placed a cap on the effectiveness of such delivery techniques. (35) Thiolated polymers or thiomers can be used to solve this essential issue. When interchain and intrachain disulfide bonds form during the swelling process, the polymeric drug carrier matrix's endurance can be significantly boosted. As a consequence, it is certain that the medicine will release under control over a lengthy time. Many different medication delivery methods employ this technique. (36)

Tissue engineering and regenerative medicine

Thiolated polymers are used as scaffolds in tissue engineering because of their biocompatibility, biological mimicry, and capacity to effectively support a wide range of cell types in their proliferation and differentiation. (37) Additionally, thiolated polymers like thiolated chitosan and hyaluronic acid are shown to have wound-healing properties. (38)

Metal binding property

Metal ion binding is a well-known property of thiols. Wilson illness is treated with substances like penicillamine and 2,3-dimercapto1-propanol due to their copper(I) binding properties. (39) The features of thiolated polymers for binding metal ions are relatively strong and quick. It has been shown that thiolated polyacrylates inhibit chymotrypsin, a protease that needs zinc ions as a co-factor. (40) Most proteolytic enzymes must have divalent cations present as cofactors in order to maintain structural integrity, which is necessary for enzymatic activity. Trypsin and -chymotrypsin are Ca2+-dependent serine proteases that are inhibited by calcium deficit. The degree of Zn2+ deficiency also affects the activity of carboxypeptidase A and cytosolic leucine aminopeptidase. (41)

WHY ‘‘Thiolated Polymers’’?

Thiomers may be useful for non-invasive medicine distribution through the mouth, eyes, nose, buccal, and vaginal channels due to their unique properties. Thiomers may be useful in tissue engineering and regenerative medicine. Thiomers can be taken orally as a solution or as crushed tablets. Thiomer-based micro- and nanoparticles are being researched. In 2012, "preactivated" or "S-protected" thiomers, a new generation of thiomers, were created. (42) Preactivated thiomers, as opposed to first-generation thiomers, are more mucoadhesive and permeability- and oxidation-resistant. (43) In diverse preparations for various routes, various polymers that have been altered by the addition of thiol groups are used. When these modified polymers are utilised, the plasma drug levels are increased. The mucoadhesive properties of the polymers are strengthened by the addition of thiol groups, which leads to polymers and, eventually, dosage forms with a number of additional attributes such taste muffling, improved permeability, delayed release, decreased pain, and patient compliance. When compared to conventional polymers, thiolated polymers or thiomers have free thiol groups on the polymer moiety, which improves mucoadhesion. Thiomers, which have side chains and include thiol groups, are the most significant mucoadhesive polymers. These are commonly employed in the creation

of various drug delivery systems and provide a number of benefits over systems based on polymers. (44)

APPLICATIONS

Active Pharmaceutical Ingredient

In general, mucoadhesive polymers are effective in treating conditions involving dry mucosal surfaces. In situations of dry mouth, dry eye, and dry vagina syndrome, lubricating mucoadhesive polymers are a potent therapy option for restoring physiological conditions by substituting missing mucus. As a result, they can reduce chewing issues, speed up tear-film break-up, and reduce local itch or irritation. Thiomers have substantially stronger mucoadhesive and in situ gelling characteristics than non-thiolated polymers, which makes them more effective. (45)

The symptoms of dry eye syndrome (DES) are frequently treated topically using biopolymers such carboxymethyl-cellulose or hyaluronic acids. These lubricants must be periodically infused since they have short resident times. Over the last ten years, chitosan-N-acetylcysteine (C-NAC) has been investigated in a number of research investigations for the treatment of DES because thiolated chitosans have high adherence to biological surfaces. Significant reductions in DES symptoms were seen in these studies. For instance, a controlled randomised double-blind trial showed that corneal damage may be reduced in >60% of patients and that there was a considerable increase in tear film thickness lasting for 24 hours following a single instillation (46).

Drug Delivery System

Thiolated polymers can prolong the mucosal residence duration of many kinds of drug delivery systems due to their mucoadhesive qualities. As a result, APIs have a longer-lasting local therapeutic impact, and systemic drug delivery has more time to absorb drugs from mucosal membranes. Additionally, they are a flexible and effective drug delivery method because to their in-situ gelling, permeability improvement, efflux pump suppression, and enzyme inhibition capabilities.

Thiomers' high degree of adaptability and flexibility, which enables the best design for each medication and delivery purpose, is detrimental from an industrial perspective. Fewer new excipients than new pharmaceuticals are registered each year due to the large expenditure needed for their development as well as the protracted development procedure. New excipients must show widespread application for a variety of medications in order to justify these expensive and time-consuming developments. (47)

Theranostics: Photodynamic and Photothermal Therapy

A metal that turns electromagnetic energy into heat (through the surface plasmon resonance phenomenon) and either a photosensitizer that produces reactive oxygen species (ROS) or a photosensitizer that promotes the creation of reactive oxygen species (ROS) are used in photodynamic and photothermal treatment. ROS or heat are utilised in both situations to destroy specific cells. The gold nanorod (GNR) system, which is synthesised by seed-mediated growth assisted by cetyltrimethylammonium bromide (CTAB), is the nanoparticular system for these treatments that has received the most research. Because of its high unspecific cytotoxicity, the utilisation of CTAB-linked GNR is restricted. (48)

Tissue Engineering

In addition to transplantation, tissue engineering provides a different approach for treating organ and tissue loss or injury. A biocompatible 3D matrix is used to seed cells from the patient, a genetically unrelated person, or an animal species. Bioactive chemicals can be added to this scaffold to enhance cellular activity and the development of the desired tissues. (49) The polymer matrix should be able to alter the chemical ligand for cell attachment while maintaining its physical form until the tissue is fully formed. It should also have hydration properties with characteristics comparable to those of the tissue, an interconnected microstructure to promote nutrient and oxygen transport, and the capacity to modify the chemical ligand for cell attachment. (50) The ability of thiomers to regulate medication release has been found to be useful in tissue engineering. For instance, Vogt et al. produced a light-activated, NO-releasing nanofibrous matrix using thiolated gelatin. This thiomer may be helpful in substituting traditional gelatin in tissue engineering since no release was accurately regulated and antibacterial efficacy against S. aureus was shown. (51)

Wound Treatment

Treatment for chronic wounds requires extensive medical intervention and is extremely expensive due to the high incidence of diabetes and ageing populations. Various techniques have been devised to speed up and lessen the discomfort of the wound healing process. (52) For instance, researchers looked at the effects of chitosan-N-acetylcysteine on corneal tissue after monocular epithelial debridement in rabbits. The thiolated chitosan group saw a markedly faster rate of wound healing as compared to the placebo group given phosphate buffered saline as a treatment. The results of the study indicate that although the underlying mechanism is not completely understood, it

may be connected to the well-known effects of unmodified chitosan on wound healing. No statistically significant change in cell migration or viability between thiolated chitosan and its parent polymer was found in in vitro investigations on fibroblasts. (53) Furthermore, it is essential for wound healing that antibacterial activity continues to fight infection. In order to achieve a sustained in vitro release of this polypeptide with broad-spectrum antibacterial action over 20 days, Trp-rich peptide (PSI) was attached to chitosan cysteine through disulfide formation. After 21 days, however, there was no discernible difference in the way mice's wounds healed since both hydrogels loaded with PSI based on thiolated chitosan and pure chitosan had developed typical epidermal-dermal layer structures. (54)

Coating Material

Coating with thiolated chitosan to alter surface characteristics is a viable method to prevent bacterial adherence and the generation of biofilm on fouling-prone materials. As an illustration, Michael addition was used to link carboxymethyl chitosan-4-thiobutylamidine on tannic acid that contained maleimido and was anchored on stainless steel. Because of this, thiolated chitosan-coated surfaces displayed 70% less protein adsorption and 91% less bacterial adherence whencompared to stainless steel treated with maleimido-containing tannic acid. (55) It is possible to link the decreased antifouling to an increase in surface hydrophilicity since protein adsorption and bacterial adhesion are correlated with surface hydrophobicity. These results, however, did not allow for any inferences regarding the thiol moiety's anti-adhesive activity. (56) In the process of creating a polymer film that would be used to package food, pegylated chitosan-4-thiobutylamidine was also researched. Because a more transparent film was created, the thiol-modified film had improved compatibility with polyethylene and antibacterial activity that the original chitosan lacked. (57)

Cosmetics

Cosmetics made from thiolated chitosans for hair, eyelashes, and eyebrow treatments (styling gels, mending agents, colouring agents, detergents, and coating), nail polishes, make-up, and antiperspirants are particularly alluring because of the thiol-bearing proteins in hair, nails, and skin. (58) Thus, by forming covalent disulfide bonds with the cysteine subunits of these surface proteins, thiolated chitosans demonstrate exceptional adherence, stabilising the product after application without spreading or dissolving. (59) As a film-forming, skin-conditioning and skin-protecting agent, chitosan is a thiolated compound. There is just one thiolate chitosant documented in the CosIng database, which is maintained by the European Commission. This thiolated chitosan has a 40% more binding capacity for Ni2+ ions than unmodified chitosan, according to a patent application for it. (60)

Water Treatment

In recent years, drinking water contamination has increased in frequency. Due to their physiochemical, mechanical, and biological capabilities for complexing and adsorbing inorganic contaminants, thiolated chitosans have a significant potential for application in the remediation of drinking water. Despite this, it might be challenging to compare different research because the conditions for the experiments are not always the same. (61) Additionally, the colorimetric measurement of Hg2+ was carried out using the complexation capability of chitosan-SH. With a detection limit of 0.465 ppb by naked eye, considerably below the WHO's upper limit of Hg2+ in drinking water of 6 ppb, the developed sensor displayed quick response, strong selectivity, and high sensitivity. (62) Thiolated chitosans are being studied for the treatment of organic contaminants in wastewater in addition to inorganic pollutants. Recently, a composite made of chitosan and Fe3O4 that is thiolated was created in order to immobilise laccase and decolorize the textile dyes Acid Blue 74 and Reactive Blue 171. Through the creation of disulfide bonds, the enzyme was firmly attached to the composite and maintained its high decolorization activity throughout time. (63)

CONCLUSION

Thiolated chitosans have a high future potential due to the wide range of sulfhydryl ligands that can be covalently attached to the polymeric backbone. Because of this variety, specific properties can be adjusted on the fly, and new functions can be added. Because thiol/disulfide exchange reactions and disulfide formation are directed by opposite charges in their surroundings attracting each other and bringing reactive sulphur species close to each other, charged ligands in particular will likely be used to a greater extent in the years to come. These ligands will enable the formation of more specific disulfide bonds between thiol or disulfide. Less reactive S-protected thiols are another class of sulfhydryl ligands that will undoubtedly shape the future landscape of thiolated chitosans. So far, thiol groups on chitosan have been S-protected via disulfide bonds formed with mercaptopyridine analogues. The disulfide bond is strong due to the electron-drawing effect of the pyridine's -system. Thiol groups on chitosan, on the other hand, are less reactive because they are S-protected by disulfide bonds formed with sulfhydryl ligands that do not have an electron-withdrawing effect, such as cysteine or N-acetylcysteine.

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CONSENT FOR PUBLICATION
The authors declare no conflict of interest.

COMPETING INTERESTS
The authors declare that they have no competing interests.

REFERENCES
[1] Kumar N, Pahuja S, Sharma R. Pharmaceutical Polymers-A Review. International Journal of Drug Delivery Technology. 2019 Jan 21;9(01):27-33.
[2] https://www.sciencenewsforstudents.org/article/explainer-what-are-polymers
[3] Odian G. Principles of Polymerization. John Wiley & Sons. Inc.: Hoboken, NJ. 2004.
[4] Flory PJ. Principles of polymer chemistry. Cornell University Press; 1953.
[5] Hanif M, Zaman M, Qureshi S. Thiomers: a blessing to evaluating era of pharmaceuticals. International Journal of Polymer Science. 2015 Jan 1;2015.
[6] Laffleur F, Bernkop-Schnürch A. Thiomers: promising platform for macromolecular drug delivery. Future medicinal chemistry. 2012 Nov;4(17):2205-16.
[7] Leichner C, Jelkmann M, Bernkop-Schnürch A. Thiolated polymers: Bioinspired polymers utilizing one of the most important bridging structures in nature. Advanced drug delivery reviews. 2019 Nov 1;151:191-221.
[8] Bernkop-Schnürch A, Clausen AE, Hnatyszyn M. Thiolated polymers: synthesis and in vitro evaluation of polymer–cysteamine conjugates. International journal of pharmaceutics. 2001 Sep 11;226(1-2):185-94.
[9] Menzel C, Silbernagl J, Laffleur F, Leichner C, Jelkmann M, Huck CW, Hussain S, Bernkop-Schnürch A. 2, 2' Dithiodinicotinyl ligands: Key to more reactive thiomers. International journal of pharmaceutics. 2016 Apr 30;503(1-2):199-206.
[10] Wilson JM, Bayer RJ, Hupe DJ. Structure-reactivity correlations for the thiol-disulfide interchange reaction. Journal of the American Chemical Society. 1977 Nov;99(24):7922-6.
[11] Nagy P. Kinetics and mechanisms of thiol–disulfide exchange covering direct substitution and thiol oxidation-mediated pathways. Antioxidants & redox signaling. 2013 May 1;18(13):1623-41.
[12] Tajc SG, Tolbert BS, Basavappa R, Miller BL. Direct determination of thiol p K a by isothermal titration microcalorimetry. Journal of the American Chemical Society. 2004 Sep 1;126(34):10508-9.
[13] Wang X, Mei Z, Wang Y, Tang L. Comparison of four methods for the biofunctionalization of gold nanorods by the introduction of sulfhydryl groups to antibodies. Beilstein journal of nanotechnology. 2017 Feb 6;8(1):372-80.
[14] Roldo M, Hornof M, Caliceti P, Bernkop-Schnürch A. Mucoadhesive thiolated chitosans as platforms for oral controlled drug delivery: synthesis and in vitro evaluation. European Journal of Pharmaceutics and Biopharmaceutics. 2004 Jan 1;57(1):115-21.
[15] Hauptstein S, Bonengel S, Griessinger J, Bernkop-Schnürch A. Synthesis and characterization of pH tolerant and mucoadhesive (thiol–polyethylene glycol) chitosan graft polymer for drug delivery. Journal of pharmaceutical sciences. 2014 Feb 1;103(2):594-601.
[16] Yadav S, Ahuja M, Kumar A, Kaur H. Gellan–thioglycolic acid conjugate: Synthesis, characterization and evaluation as mucoadhesive polymer. Carbohydrate polymers. 2014 Jan 2;99:601-7.
[17] Deepak K, Kumar MS, Mahadevan N. Thiolated chitosan: modified advanced generation of mucoadhesive polymers. International Journal of Recent Advance in Pharmaceutical Research. 2012;2(3):31-41.
[18] Singh I, Rana V. Enhancement of mucoadhesive property of polymers for drug delivery applications. Reviews of Adhesion and Adhesives. 2013 Apr 1;1(2):271-90.
[19] Smart JD. The basics and underlying mechanisms of mucoadhesion. Advanced drug delivery reviews. 2005 Nov 3;57(11):1556-68.
[20] Leitner VM, Walker GF, Bernkop-Schnürch A. Thiolated polymers: evidence for the formation of disulphide bonds with mucus glycoproteins. European Journal of Pharmaceutics and Biopharmaceutics. 2003 Sep 1;56(2):207-14.
[21] https://www.mdpi.com/polymers/polymers-1201803/article_deploy/html/images/polyme-12-01803-g001-550.jpg
[22] Wertz PW, Downing DT. Stratum corneum: biological and biochemical considerations. Transdermal drug delivery. 1989;35:1-22.
[23] Grießinger JA, Bonengel S, Partenhauser A, Ijaz M, Bernkop-Schnürch A. Thiolated polymers: evaluation of their potential as dermoadhesive excipients. Drug development and industrial pharmacy. 2017 Feb 1;43(2):204-12.
[24] Wolfram LJ, Underwood DL. The equilibrium between the disulfide linkage in hair keratin and sulfite or mercaptan. Textile Research Journal. 1966 Nov;36(11):947-53.

[25] Zhang Y, Zhou S, Deng F, Chen X, Wang X, Wang Y, Zhang H, Dai W, He B, Zhang Q, Wang X. The function and mechanism of preactivated thiomers in triggering epithelial tight junctions opening. European Journal of Pharmaceutics and Biopharmaceutics. 2018 Dec 1;133:188-99.

[26] Sebé-Pedrós A, Roger AJ, Lang FB, King N, Ruiz-Trillo I. Ancient origin of the integrin-mediated adhesion and signaling machinery. Proceedings of the National Academy of Sciences. 2010 Jun 1;107(22):10142-7.

[27] Clausen, A. E., Kast, C. E., & Bernkop-Schnürch, A. (2002). The role of glutathione in the permeation enhancing effect of thiolated polymers. Pharmaceutical research, 19(5), 602-608.

[28] Moreno-Merlo F, Nicklee T, Hedley DW. Association between tissue hypoxia and elevated non-protein sulphydryl concentrations in human cervical carcinoma xenografts. British journal of cancer. 1999 Nov;81(6):989-93.

[29] Glass JJ, Li Y, De Rose R, Johnston AP, Czuba EI, Khor SY, Quinn JF, Whittaker MR, Davis TP, Kent SJ. Thiol-reactive star polymers display enhanced association with distinct human blood components. ACS applied materials & interfaces. 2017 Apr 12;9(14):12182-94.

[30] Esmaeilzadeh P, Menzel M, Groth T. Cyclic redox-mediated switching of surface properties of thiolated polysaccharide multilayers and its effect on fibroblast adhesion. ACS applied materials & interfaces. 2018 Aug 29;10(37):31168-77.

[31] Bernkop-Schnürch A, Schwarz V, Steininger S. Polymers with thiol groups: a new generation of mucoadhesive polymers?. Pharmaceutical research. 1999 Jun;16(6):876-81.

[32] Werle M, Hoffer M. Glutathione and thiolated chitosan inhibit multidrug resistance P-glycoprotein activity in excised small intestine. Journal of controlled release. 2006 Mar 10;111(1-2):41-6.

[33] Grabovac V, Laffleur F, Bernkop-Schnürch A. Thiomers: Influence of molecular mass and thiol group content of poly (acrylic acid) on efflux pump inhibition. International journal of pharmaceutics. 2015 Sep 30;493(1-2):374-9.

[34] Clausen AE, Kast CE, Bernkop-Schnürch A. The role of glutathione in the permeation enhancing effect of thiolated polymers. Pharmaceutical research. 2002 May;19(5):602-8.

[35] Langoth N, Kalbe J, Bernkop-Schnürch A. Development of a mucoadhesive and permeation enhancing buccal delivery system for PACAP (pituitary adenylate cyclase-activating polypeptide). International journal of pharmaceutics. 2005 May 30;296(1-2):103-11.

[36] Bernkop-Schnürch A, Scholler S, Biebel RG. Development of controlled drug release systems based on thiolated polymers. Journal of controlled release. 2000 May 3;66(1):39-48.

[37] Mishra BJ, Kaul A, Trivedi P. L-Cysteine conjugated poly L-lactide nanoparticles containing 5-fluorouracil: formulation, characterization, release and uptake by tissues in vivo. Drug delivery. 2015 Feb 17;22(2):214-22.

[38] Gajendiran M, Rhee JS, Kim K. Recent developments in thiolated polymeric hydrogels for tissue engineering applications. Tissue Engineering Part B: Reviews. 2018 Feb 1;24(1):66-74.

[39] Zahir-Jouzdani F, Mahbod M, Soleimani M, Vakhshiteh F, Arefian E, Shahosseini S, Dinarvand R, Atyabi F. Chitosan and thiolated chitosan: Novel therapeutic approach for preventing corneal haze after chemical injuries. Carbohydrate polymers. 2018 Jan 1;179:42-9.

[40] Smirnova J, Kabin E, Järving I, Bragina O, Tõugu V, Plitz T, Palumaa P. Copper (I)-binding properties of de-coppering drugs for the treatment of Wilson disease. α-Lipoic acid as a potential anti-copper agent. Scientific reports. 2018 Jan 23;8(1):1-9.

[41] Bernkop-Schnürch A, Thaler SC. Polycarbophil–cysteine conjugates as platforms for oral polypeptide delivery systems. Journal of pharmaceutical sciences. 2000 Jul 1;89(7):901-9.

[42] Madsen F, Peppas NA. Complexation graft copolymer networks: swelling properties, calcium binding and proteolytic enzyme inhibition. Biomaterials. 1999 Sep 1;20(18):1701-8.

[43] Iqbal J, Shahnaz G, Dünnhaupt S, Müller C, Hintzen F, Bernkop-Schnürch A. Preactivated thiomers as mucoadhesive polymers for drug delivery. Biomaterials. 2012 Feb 1;33(5):1528-35.

[44] Ijaz M, Bernkop-Schnürch A. Preactivated thiomers: their role in drug delivery. Expert opinion on drug delivery. 2015 Aug 3;12(8):1269-81.

[45] Puri V, Sharma A, Kumar P, Singh I. Thiolation of biopolymers for developing drug delivery systems with enhanced mechanical and mucoadhesive properties: A review. Polymers. 2020 Aug;12(8):1803.

[46] Partenhauser, A., & Bernkop-Schnürch, A. (2016). Mucoadhesive polymers in the treatment of dry X syndrome. Drug discovery today, 21(7), 1051-1062.

[47] Schmidl D, Werkmeister R, Kaya S, Unterhuber A, Witkowska KJ, Baumgartner R, Höller S, O'Rourke M, Peterson W, Wolter A, Prinz M. A controlled, randomized double-blind study to evaluate the safety

and efficacy of chitosan-N-acetylcysteine for the treatment of dry eye syndrome. Journal of Ocular Pharmacology and Therapeutics. 2017 Jun 1;33(5):375-82.

[48] Shah KU, Shah SU, Dilawar N, Khan GM, Gibaud S. Thiomers and their potential applications in drug delivery. Expert opinion on drug delivery. 2017 May 4;14(5):601-10.

[49] Almada M, Leal-Martínez BH, Hassan N, Kogan MJ, Burboa MG, Topete A, Valdez MA, Juárez J. Photothermal conversion efficiency and cytotoxic effect of gold nanorods stabilized with chitosan, alginate and poly (vinyl alcohol). Materials Science and Engineering: C. 2017 Aug 1;77:583-93.

[50] Federer C, Kurpiers M, Bernkop-Schnüŕch A. Thiolated chitosans: a multi-talented class of polymers for various applications. Biomacromolecules. 2020 Jun 22;22(1):24-56.

[51] Jiao J, Huang J, Zhang Z. Hydrogels based on chitosan in tissue regeneration: How do they work? A mini review. Journal of Applied Polymer Science. 2019 Apr 5;136(13):47235.

[52] Vogt C, Xing Q, He W, Li B, Frost MC, Zhao F. Fabrication and characterization of a nitric oxide-releasing nanofibrous gelatin matrix. Biomacromolecules. 2013 Aug 12;14(8):2521-30.

[53] Beconcini D, Fabiano A, Di Stefano R, Macedo MH, Felice F, Zambito Y, Sarmento B. Cherry extract from Prunus avium L. to improve the resistance of endothelial cells to oxidative stress: Mucoadhesive chitosan vs. poly (lactic-co-glycolic acid) nanoparticles. International journal of molecular sciences. 2019 Jan;20(7):1759.

[54] Huang L, Zhu Z, Wu D, Gan W, Zhu S, Li W, Tian J, Li L, Zhou C, Lu L. Antibacterial poly (ethylene glycol) diacrylate/chitosan hydrogels enhance mechanical adhesiveness and promote skin regeneration. Carbohydrate polymers. 2019 Dec 1;225:115110.

[55] Xu LQ, Pranantyo D, Neoh KG, Kang ET, Fu GD. Thiol reactive maleimido-containing tannic acid for the bioinspired surface anchoring and post-functionalization of antifouling coatings. ACS Sustainable Chemistry & Engineering. 2016 Aug 1;4(8):4264-72.

[56] Cunliffe D, Smart CA, Alexander C, Vulfson EN. Bacterial adhesion at synthetic surfaces. Applied and environmental microbiology. 1999 Nov 1;65(11):4995-5002.

[57] Davidovich-Pinhas M, Danin-Poleg Y, Kashi Y, Bianco-Peled H. Modified chitosan: A step toward improving the properties of antibacterial food packages. Food Packaging and Shelf Life. 2014 Jun 1;1(2):160-9.

[58] Leierer J, inventor; THIOMATRIX FORSCHUNGS-UND BERATUNGS GmbH, Croma Pharma GmbH, assignee. Mucoadhesive polymers having vitamin B partial structures. United States patent US 10,639,377. 2020 May 5.

[59] Prinz M, inventor; MucoBiomer Biotechnologische Forschungs-u Entwicklungs Ges mbH, assignee. Chitosan-thio-amidine conjugates and their cosmetic as well as pharmaceutic use. United States patent US 7,053,068. 2006 May 30.

[60] Bal W, Zawisza I, Fraczyk T, inventors; INSTYTUT BIOCHEMII I BIOFIZYKI POLSKIEJ AKADEMII NAUK, assignee. Method for synthesis of a biopolymer derivative, a biopolymer derivative and its use. United States patent application US 15/118,641. 2017 Mar 2.

[61] Singh P, Chauhan K, Priya V, Singhal RK. A greener approach for impressive removal of As (III)/As (V) from an ultra-low concentration using a highly efficient chitosan thiomer as a new adsorbent. RSC advances. 2016;6(69):64946-61.

[62] Chauhan K, Singh P, Singhal RK. New chitosan–thiomer: an efficient colorimetric sensor and effective sorbent for mercury at ultralow concentration. ACS applied materials & interfaces. 2015 Dec 2;7(47):26069-78.

[63] Ulu A, Birhanli E, Boran F, Köytepe S, Yesilada O, Ateş B. Laccase-conjugated thiolated chitosan-Fe3O4 hybrid composite for biocatalytic degradation of organic dyes. International journal of biological macromolecules. 2020 May 1;150:871-84.