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Review Article

Thiolation of Polymers; A re-oriented approach towards targeted drug delivery.

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Abstract

Targeted drug delivery remains a challenge for the pharmaceutical scientists due to less solubility of API and less compatibility and contact time of excipients with mucous membrane. Modification in the composition of excipients by addition of different functional groups can be used to overcome the problems. Addition of thiol group by different methods can increase the mucoadhesion, solubility, stability in acidic medium and contact time of dosage forms. Resulting thiol containing polymers make a disulfide bonds with the mucous membrane. Review contains a brief account on introduction to thiomers, polymers used in thiolation, S-protected preactivated thiomers, dosage forms including micro and nanoparticles, microgels and transdermal patches comprising thiolated polymers and description of different preparatory methods of thiolated polymers. Additionally, the review also focused In-vivo studies performed on mucosal membrane of animals to ensure the improved permeation, efflux pump inhibition and controlled release effect of thiomers.

Keywords: Thiolation, Thiomers, mucoadhesion , thiolated polymers, biodegradable polymers,

Introduction

Mucoadhesion, describes the attachment of any natural, synthetic or semi synthetic macromolecule to a mucous membrane by means of interactive forces (Smart 2014). Mechanism of mucoadhesion prolongs the residence time of dosage forms resulting in upgraded drug bioavailability. Transport of high molecular weight molecules across the membrane can be increased by developing these systems with some suitable material such as a biodegradable polymer (Rahamatullah Shaikh *et al.* 2011). These polymers are hydrophilic in nature and on absorbing water provide sufficient adhesion to the drug delivery system. Strong hydrogen bond forming groups such as hydroxyl, amide or carboxylic groups are present in all mucoadhesive polymers. The process of mucoadhesion is highly influenced by not only some polymer characteristics such as hydrophophilicity, and optimum molecular weight but also several attempts have been made to increase several attemtps have been made to increase the mucoadhesion of dosage forms such as attachment of Polyethylene glycol (PEG), development of hydrogels (Gajendiran *et al.* 2018) and creating sustained hydration process (Bernkop-Schnürch 2005). But all these formulations were based on weak non-covalent interactions such as hydrogen bonding, Vander Waals's forces or ionic interactions. Thus, provide only limited adhesion to the carrier system. In order to overcome these issues, a new generation of mucoadhesive polymers, called thiolated polymers were developed. These sulfure containing molecules commonly termed as "Thiolated polymers" or "Thiomers" ; contain thiol groups (Liang *et al.* 2018) along with the polymeric back bone which strongly interact with the mucin of mucous membrane to provide enhanced mucoadhesion (Figure. 1) (Kaur *et al.* 2012; Verheul *et al.* 2010; Zambito *et al.* 2013).

Better drug permeation, added cohesiveness, increased resistance to efflux inhibition, higher bioavailability, less dosing frequency,

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and ultimately increased patient compliance are the key features of thiomers. Moreover, Shika proved that thiolation does not affect the non-irritant nature of the original polymers (Yadav *et al.* 2014).

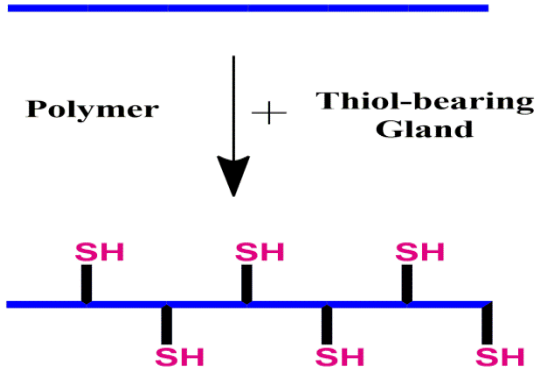

Figure 1: Thiomer interaction with the mucus membrane.

To date, many polymers have been thiolated such as chitosan (Anitha *et al.* 2011; Lee *et al.* 2007; Shahnaz *et al.* 2012), sodium alginate (Davidovich-Pinhas *et al.* 2009), hydroxyl ethyl cellulose (Rahmat *et al.* 2013), polycarbophil (Cevher *et al.* 2008), carboxy methyl cellulose (CMC) (Clausen and Bernkop-Schnürch 2001), gallen gum (YadavAhuja *et al.* 2014), xyloglucan (Bhalekar *et al.* 2013), polyacrylic acid (Sarti *et al.* 2012), Xanthan gum (Bhatia *et al.* 2015) etc., and used to develop many formulations. The very first product containing a thiomer Lacrimera® by Croma pharma has reached the market of USA and Canada in early 2015. They exhibit mucoadhesion, permeation enhancement, and efflux pump inhibition effect. In addition, they are biodegradable and their toxicity profile is very low (Bonengel and Bernkop-Schnürch 2014).

TYPES OF THIOMERS

There are two types of thiomers reported in the literature i.e Cationic thiomers and anionic thiomers. Figure 2 illustrates classification of thiomers.

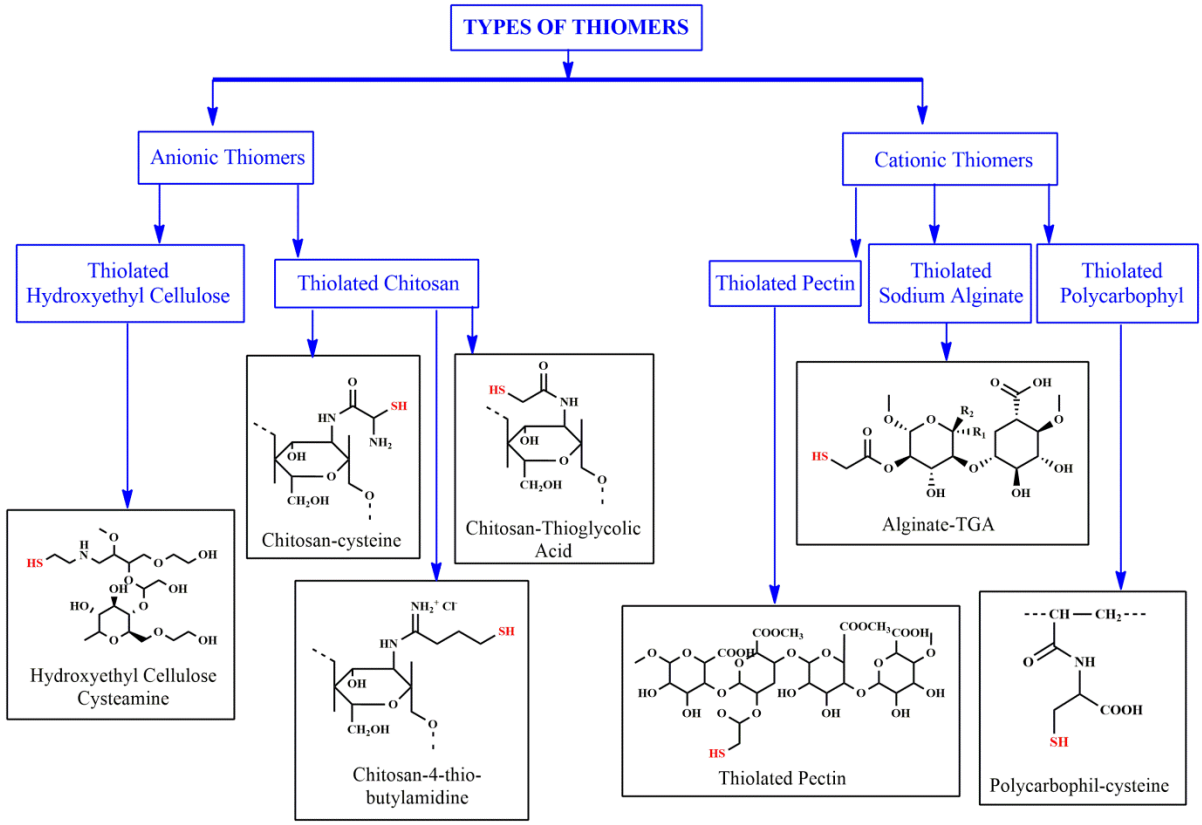

Figure 2: Schematic diagram showing types of thiomers.

Cationic thiomers

Chitosan derivatives

Chitosan is obtained by alkaline deacetylation of chitin, an abundantly found natural polysaccharide (Figure. 3). It is a polymer of glucosamine and N-acetyl glucosamine subunits, extensively used for mucoadhesion due to its low toxicity profile and high adhesive power with the mucin layer (Ghanbarzadeh and Almasi 2013).

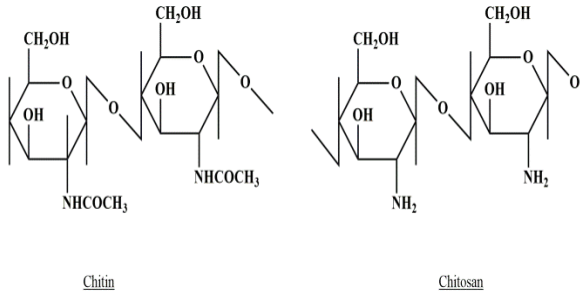

Figure 3: Structure of Chitin and Chitosan molecules.

The positive charge of chitosan form strong bond with the negative acidic group of mucin and provide adhesion and high permeation to the system (Hermans *et al.* 2014). So by the introduction of thiol group to chitosan its mucoadhesion has been improved via disulfide bond formation (Hanif *et al.* 2015). Primary amino group at position 2 of the glucosamine unit is where the thiol group is attached. The amino group reacts with the carboxylic functional group of the sulfhydryl containing reagent (AnithaDeepa et al. 2011; LeeZhang et al. 2007; ShahnazVetter et al. 2012). Many thiolated conjugates of chitosan have been prepared and proved for enhanced mucoadhesion. Chitosan react with thioglycolic aicd (TGA) (CevherSensoy *et al.* 2008) and cysteine via amide bond formation while an amidine bond is formed with 2-iminothioleine (Bernkop-Schnürch *et al.* 2003).

Thiolated Hydroxyethyl cellulose

A problem with thiolated chitosan is its precipitation at pH above 6.5. To overcome this problem another cationic polymer hydroxyethyl cellulose (HEC) was modified by the process of thiolation. In a study HEC-cystamine was reported to possess enhanced mucoadhesion and penetration effects due to the formation of disulfide bonds between the free thiol group of polymer and mucin (Rahmat *et al.* 2011). In another study nanoparticles based on thiolated HEC showed increase stability towards the enzymatic degradation. Mucoadhesion and permeation enhancing properties were significantly improved in thiolated HEC when compared to the original HEC (RahmatMüller *et al.* 2013).

Anionic thiomers

These polymers contain carboxylic group and react with the sulfhydryl group via amide bond formation in the presence of carbodiimides. When compared to the original polymers, thiomers were found to possess enhanced properties of permeation, mucoadhesion, and an improvement in efflux pump inhibition effect (Palmberger *et al.* 2015).

Polycarbophil

Polycarbophil-cysteine conjugates showed more adhesion strength as compared to the original polymer and drug release from the dosage is controlled making it more suitable to formulate a controlled release system (Albrecht *et al.* 2006; CevherSensoy *et al.* 2008).

Sodium alginate

Thiolation of sodium alginate causes an increase in the disintegration time of tablets and drug is released over an extended period of time; making it a suitable candidate for controlled release system (Jindal *et al.* 2010). In a study Kaseem. developed

microbeads of thiolated sodium alginate for the delivery of resveratrol with an aim to increase its localized effect and reported augmented mucoadhesive strength (Kassem *et al.* 2015).

Pectin

Pectin, after thiolation with cysteine and thioglycolic acid (TGA), showed improved mucoadhesion and permeation effect as compared to the unmodified polymer (Hintzen *et al.* 2012b). In a study, Sharma studied its drug release profile and reported its need in the formulation of an extended release dosage form (Sharma and Ahuja 2011).

Methods for preparation of thiomers

To develop thiol-containing polymers two methods could be employed. One is by activation of carboxylic acid moieties and the other is by esterification. In the most commonly applied method polymer is dissolved to get a clear solution. A carbodimide e.g 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) is used to activate the carboxylic acid moieties. After pH adjustment, thiol containing reagent is added and reaction is continued. Product is dialyzed against HCl and NaOH to isolate the thiolated polymer from the reaction mixture. Subsequently, obtained thiomers are then lyophilized by freeze drying and stored at 40°C. In the process of thiolation different reagents have been used like TGA, L-cysteine, 2-iminothiolane, and cysteamine (RahmatSakloetsakun *et al.* 2011; PalmbergerLaffleur *et al.* 2015; Bernkop-SchnürchHornof *et al.* 2003; CevherSensoy *et al.* 2008).

The other method is based on esterification between acidic groups of polymer and thio-alcoholic group of the reagent. Thiolation is done by reacting two moles of thiol containing reagent for each mole of polymer. Concisely specified amount of the polymer is dissolved in water to obtain a clear solution. Later, to this solution a mixture of reagent i.e. thioglycolic acid and HCL of required normality is added dropwise, and let the reaction to proceed at a constant temperature of 80°C for about two and a half hours. Finally, precipitates are obtained by adding this blend into sufficient amount of methanol. Precipitates obtained are then washed again with methanol and dried at room temperature (KassemFarid *et al.* 2015; Sharma and Ahuja 2011).

Improved characteristics of polymers after thiolation

Permeation enhancement

Biodegradable polymers e.g. chitosan was well considered as permeation enhancer polymer from years. After thiolation its permeation enhancing properties were increased as compared to unmodified chitosan (Bernkop-SchnürchHornof *et al.* 2003). Glutathione (GSH) has been known for increasing the permeation across membrane because it inhibits the protein tyrosine phosphate (PTP). Occludin, a protein in the epithelial cells involved in opening and closing of tight junction for the transport of materials. The process of dephosphorylation of Occludin in the epithelial cells is regulated by PTP and involved in closing of junction in cell membanes. The free thiol group of GSH react with the cysteine of protein to form disulfide bond. Inhibition of PTP causes an increase in phosphorylated protein that enhances the permeation (Di Colo *et al.* 2008).

Guggi and Bernkop studied chitosan-TBA (thiobutyl amide) in combination with GSH

and bromelain; another permeation enhancer, by using high molecular weight marker Fluorescein isothiocyanate dextrane. Results showed that the combination of two differently acting permeation enhancer could be a promising tool for increasing the permeation of macromolecules in a more stable manner (Guggi and Bernkop-Schnürch 2005). In another study these two strategies were combined by directly immobilizing the GSH on chitosan. These conjugates of chitosan has shown a greater permeation along with increased adherence to mucous membrane than many other thiomers (Bernkop-Schnürch *et al.* 2004a).

Efflux pump inhibitor

The absorption of substances across the cell membranes is regulated by thy efflux pumps located on the membrane. To facilitate the transportation of drugs it is necessary to overcome the hindrance presented by ABC transporters, multiple drug resistance proteins referred as plasma glycoprotein (P-gp), multidrug resistance associated proteins etc. Foger. studied P- gp inhibitory effect of thiolated chitosan which showed 2.7 fold more inhibitory effect than unchanged polymer and increased uptake of drug (Föger *et al.* 2006). Molecular weight of polymer and degree of thiolation greatly influence the efflux pump inhibition effect of the membrane transporters. Grabovac showed that higher degree of thiolation was related with the increased inhibition of efflux pumps (Grabovac *et al.* 2015). Efflux pump inhibition by sulforhodamine 101 (SR101), a multi-drug resistance associated protein (MRP2) substrate has been reportedly increased after incorporation of thiolated conjugates. The possible mechanism proposed that it may have altered the membranous structure of intestinal cells as it couldn't be up taken by the cells directly (PalmbergerLaffleur *et al.* 2015). Deni studied different conjugates of HEC cysteamine for its efflux pump inhibition effect by using rhodamine 123 which is P-gp substrate. Results showed polymer's efflux pump inhibition effect at all concentration while permeation enhancing effects are observed at higher concentration of thiol group (Rahmat *et al.* 2012).

Mucoadhesion

One of the reasons behind concept of thiolation is to intensify the mucoadhesive ability of the polymers. Various studies showed that the thiolated polymers display enhanced mucoadhesion as compared to actual polymers. Iimmobilization of thiol groups to the polymer increases the adhesive strength of the polymer which primarily depends upon the concentration and nature of the thiol containing moiety (Storha *et al.* 2013). Thiomers mimic the natural mechanism of glycoproteins attached to the mucosal membrane by forming disulphide bonds. Mucous membrane lines many internal organs like respiratory tract, GIT etc. Major portion of the mucous membrane is mucin that are large and complex glycoproteins (Johansson *et al.* 2013). Mucin contain cysteine rich domains all over it, which react with the thiol groups of the polymer via disulfide bond formation. These disulfide bonds are covalent in nature and hence this connection is stronger than that provided by the native polymers (Davidovich-PinhasHarari *et al.* 2009; Schmitz *et al.* 2008). Biodegradable polymers adhere to the mucosa and increase the residence time of dosage form on the site of absorption. Many of these polymers are undergone thiolation and proved to provide comparatively enhanced mucoadhesion

(Table 1). (Iqbal *et al.* 2012; Kast and Bernkop-Schnürch 2001; Mahajan *et al.* 2013; AlbrechtGreindl *et al.* 2006; Bernkop-Schnürch *et al.* 2004b; Bernkop-SchnürchHornof *et al.* 2003; SchmitzGrabovac *et al.* 2008; Bernkop-SchnürchGuggi *et al.* 2004a)

M.Bhalekar performed *in vivo* mucoadhesion studies on rabbit intestinal mucosa and reported X-ray results at different time intervals that confirmed the adhesion for longer time (BhalekarSonawane *et al.* 2013).

Table 1: Increase in the mucoadhesion strength of different polymers

Polymer conjugates	Unmodified polymers	Type of polymer	Thiol bearing reagent	Increase in Mucoadhesion
Chitosan-glutathione	Chitosan	Cationic	Gluthathione	55 folds
Chitosan-TGA	Chitosan	Cationic	Thioglycolic acid	10 folds
PCP-cysteien	Polycarbophil	Cationic	L-cysteine	1.9 folds
Chitosan-TBA	Chitosan	Cationic	2-iminiothiolane	140 folds
PAA-cysteine	Poly acrylic acid	Anionic	Cysteine	452 folds
Chitosan-NAC	Chitosan	Cationic	N-acetyl cysteine	50 folds
Xyloglucan cysteine	Xyloglucan		L-cysteine	1.5 folds

Controlled drug release

Thiolated polymers are studied for their possible use in controlled release formulations because the drug can be released slowly from these polymers. Cysteine conjugates of Carboxymethyl guar gum (CMG) and Carboxymethyl starch (CMS) showed initial slow release and then 88% and 94% cumulative release over a period of 6 hours respectively, confirming the potential of thiomers to be used as release controlling material. Disulfide bond formation after swelling within the polymer reduced the amount of drug releasing from the polymer (Landge *et al.* 2012). Poly (styrene sulfonic acid-co-maleic acid) PSSA.MA cysteamine conjugates has been used for ocular delivery of drugs as they exhibit controlled release of drug which increases the bioavailability of drug with minimum dosing frequency (Mahmood *et al.* 2015a). Vitamin B12 has short residence time and low permeation across GIT mucosa. It was incorporated into thiolated polyacrylic acid based microparticles and studied for its in vitro release. In this study Sarti. Reported burst release of drug followed by the slow gradual release (SartiIqbal *et al.* 2012).

In situ Gelation

Rapid clearance of the dosage form from the site of administration decreases the plasma concentration of drug. Increasing the viscosity of formulation will help to reduce the clearance of carrier system from the site of administration (Krauland *et al.* 2005). Viscoelastic properties like viscous modulus and elastic modulus of Chitosan-TGA conjugates were evaluated at pH 5.5 and results reported a direct relation between the amount of immobilized thiol groups and in situ gelling properties of thiolated chitosan. It was due to the formation of inter and intra molecular disulphide bonds which result in a cross linked structure and chain entanglements within the polymer, hence the gel formation on absorbing water and increased viscosity of carrier system. The conjugate containing the higher content of thiol groups showed more increase in the elastic and viscous modulus as compared to others (Hornof *et al.* 2003). Likewise the study of in situ gelling properties of anionic thiomers by the oscillatory measurements

showed an increase in the formulation viscosity (Hintzen *et al.* 2012a).

Preactivated thiomers

High sensitivity of thiomers towards oxidation in aqueous environment at higher pH is a main drawback limiting their use at higher pH (IqbalShahnaz *et al.* 2012). To combat this problem, the concept of a second generation of thiomers named as preactivated thiomers was introduced based on previous covalent reactivation of resins in which thiol groups are protected by pyridyl substructures (Bonengel and Bernkop-Schnürch 2014).

This idea of thiol group protection was introduced by Dünnhaupt in 2012. They eliminated pyridyl because of its toxicity and used 6-mercaptonicotinic acid (6-MNA) instead. Initially 6-MNA is oxidized to 6, 6' DTNA (6, 6' Dithiodinicotinic acid) which is its dimer and then the free thiol group of the thiomer covalently attached with the thiol group of 6, 6'DTNA via the formation of disulfide bonds with in the polymer. This new S-protected thiomer possess enhanced mucoadhesion , efflux inhibitory and permeation effects (Dünnhaupt *et al.* 2012). Buccal formulation based on preactivated pectin-cysteine were prepared and examined for their swelling behavior, mucoadhesion and release characteristics in comparison with the unmodified and thiolated pectin. There was an increase in viscosity of thiolated pectin and its derivative by 92 and 4958 times respectively (Hauptstein *et al.* 2014). Arshad developed a thiolated polysulfonate conjugates and examined its permeation enhancing and mucoadhesion effect. Permeation enhancing effects were evaluated on caco cell 2 by using Na Flu and FD4. These conjugates showed enhanced permeation which was related to their degree of thiolation (Mahmood *et al.* 2015b).

Dosage forms

Matrix tablets

The problem associated with the matrix tablets is less mucoadhesion provided by the first generation polymers, which is improved by using thiolated polymers. Matrix tablets of Ribofluvin based on Polyacrylic acid-cysteine and Chitosan-TBA conjugates showed a noticeable increment in the mucoadhesive strength and disintegration time which made these thiomers based matrix tablets a useful approach as gastro retentive delivery system to provide the longer availability of drugs (Senyigit *et al.* 2011).

Micro and Nano particles

The use of thiolated polymers was employed to produce mucoadhesive and stable yet biodegradable microparticles without the use of any cross linker. A burst release initially has been shown but then the remaining drug is released over a longer period making them a suitable candidate for controlled drug delivery systems as compared to the primitive polymer. Reason behind this phenomena is that the polymer swells on contact with the mucosa and drug is diffused out of the polymeric network (Maculotti *et al.* 2005). Microparticles based on thiolated polyacrylic acid encapsulating the exenatide has been proved in vivo to provide good permeation via nasal administration and possess no cilliotoxic effects (Millotti *et al.* 2014). Thiomers based insulin loaded microparticles investigated in vivo and proved to possess better drug penetration ability (Deutel *et al.* 2016). Thiol-ene click reaction was used to immobilize thiol groups on nanoparticles. Mucoadhesive strength of these particles was investigated by

fluorescent labelling in porcine bladder. As the presence of ester linkages render their hydrolytic degradation but they showed a slow degradation process which implies their use in sustained release carrier systems (StorhaMun *et al.* 2013). Nanoparticles prepared by using thiolated chitosan has been investigated and proved to exhibit enhanced mucoadhesion and penetration than the nanoparticles of inherent chitosan (Saboktakin *et al.* 2011).

Gels & Microgels

Mucoadhesive nature of thiolated polymers provides an advantage to the gel forming systems. The higher mucoadhesive strength and gelling property of these formulations is due to the in situ gelation property of thiomers (CevherSensoy *et al.* 2008). Recently, Cook developed a thiol containing monomer which undergoes co-polymerization in the presence of cross linker to form microgels. These microgels have been proven to possess enhanced mucoadhesion in *ex vivo* studies and can be used to provide controlled release of drugs. The consistent release of drugs from these microgels made them a good candidate to be used in chemotherapeutics delivery (Cook *et al.* 2015).

Transdermal Patches

The use of thiolated polymers along with the transdermal technology is a new approach which may be helpful in improving the mucoadhesion of the patches. Drug permeation through transdermal patches is significantly enhanced by using thiolated chitosan and is proportional to the concentration of thiol group in the polymer (Satheeshababu and Shivakumar 2013). Transdermal patches formulated with thiolated sodium alginate has shown increased drug permeation when compared to the innate sodium alginate polymer (BK and Rao 2015).

Conclusion

Thiolation of polymers has gained its importance over past years. The introduction of thiol moiety to the polymers increased its mucoadhesive strength, cohesiveness and in situ gelling properties. Enhanced permeation and efflux pump inhibition effects have been demonstrated by many *in vitro* and *in vivo* studies. In order to uplift the phenomena of controlled release, intensify permeation, and boost mucoadhesion of the drug delivery system; thiomers are currently used in combination with numerous new technologies, and with various drugs. Nanoparticulate systems, transdermal systems, hydrogels, matrix tablets and microgels based on these thiolated polymers have far better mucoadhesive characteristics in relation to the unmodified genuine polymers. Despite their modification and increased strength, they remain biodegradable and offer no toxicity to the biological systems. All these characteristics make these thiomers a promising tool for their use as polymeric excipients for the administration of macromolecules.

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