WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 10, 622-636. Review Article ISSN 2277– 7105

APPLICATION OF THIOLATED CHITOSAN IN MUCOADHESIVE BUCCAL DRUG DELIVERY SYSTEM

Dhanashri P. Garud*, Kalyani Dalavi and Sagar Deole

Student, Department of Pharmaceutics Government College of Pharmacy, Aurangabad, India.

Article Received on
13 July 2020,
Revised on 03 August 2020,
Accepted on 23 August 2020,
DOI: 10.20959/wjpr202010-18563

***Corresponding Author**
Dhanashri P. Garud
Student, Department of Pharmaceutics Government College of Pharmacy, Aurangabad, India.

ABSTRACT

A mucoadhesive buccal film of chemically modified chitosan has been developed for controlled and prolonged drug delivery application. Chitosan is chemically modified through thiol bearing reagent leads to formation thiolated chitosan which improves mucoadhesivity. The purpose of this review article is to provide brief description of mucoadhesive buccal drug delivery system, followed by thiolated chitosan derivatives. The present article provides insights into various issues like anatomy of buccal mucosa, benefits of buccal films, theories of Mucoadhesion, thiolated chitosan derivatives and evaluation of buccal film.

KEYWORDS: Mucoadhesion, chitosan, thiolated chitosan, disulfide bonds.

INTRODUCTION

The oral route is most preferred route of administration for topical and systemic delivery of therapeutic agents. However, conventional oral administration through gastrointestinal tract results in markedly reduced drug delivery for some drug due to presence of metabolic enzyme and the first pass metabolism effect. Herein the oral mucosal administration refers alternate route of administration to a drug delivery system in which the carrier made of mucoadhesive materials which adheres to mucosa.[1]

Buccal mucosa is easily accessible and convenient site for administration of drug of choice for both local (mucosal) and systemic (transmucosal) delivery through the buccal mucosal membrane lining of the oral cavity.[2,3] Flushing action of saliva dilutes and rapidly eliminates the drug from oral cavity and which minimizes the bioavailability of the drug. For the better improvement of bioavailability and local action through the oral cavity its need to formulate

the dosage form which improves the drug retention time in the oral cavity.[4] The development of novel drug delivery system using mucoadhesive polymers become more demanding in recent years due to its targeted and controlled drug delivery. Mucoadhesion is the chemical interaction between mucin of the mucosa and natural or synthetic polymer.

Human oral cavity[3,5,6]

In humans, the oral mucosal surface area is about 100 cm2. Oral mucosa can be differentiated by three types; 1) the masticatory mucosa which is 25% of total oral mucosa having a thickness of 100–200 µm and present in gingival and hard palate. 2) The lining mucosa covers 60% of the total area and has a thickness of 500–800 and present on lips, cheeks, soft palate, lower surface of tongue and floor of oral cavity 3) Specialized mucosa covers 15% of total mucosa and found in dorsum of tongue.

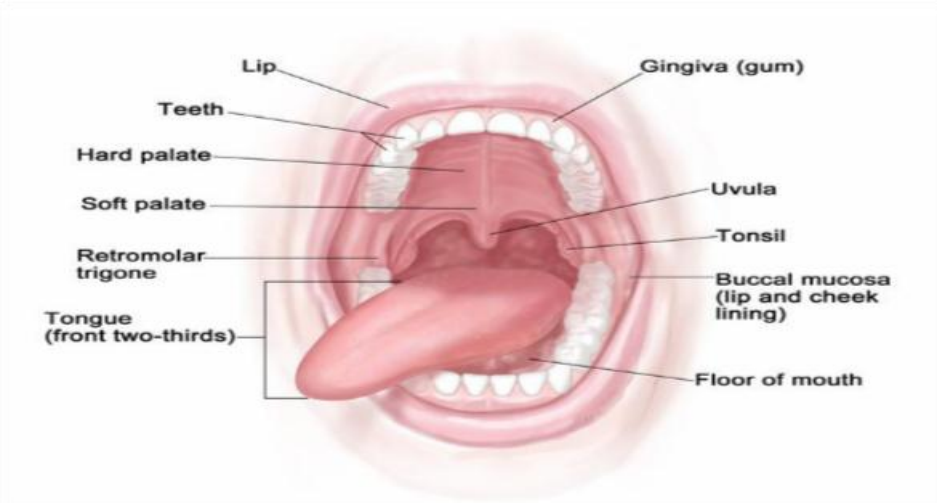


Fig. Mucosal region of mouth.

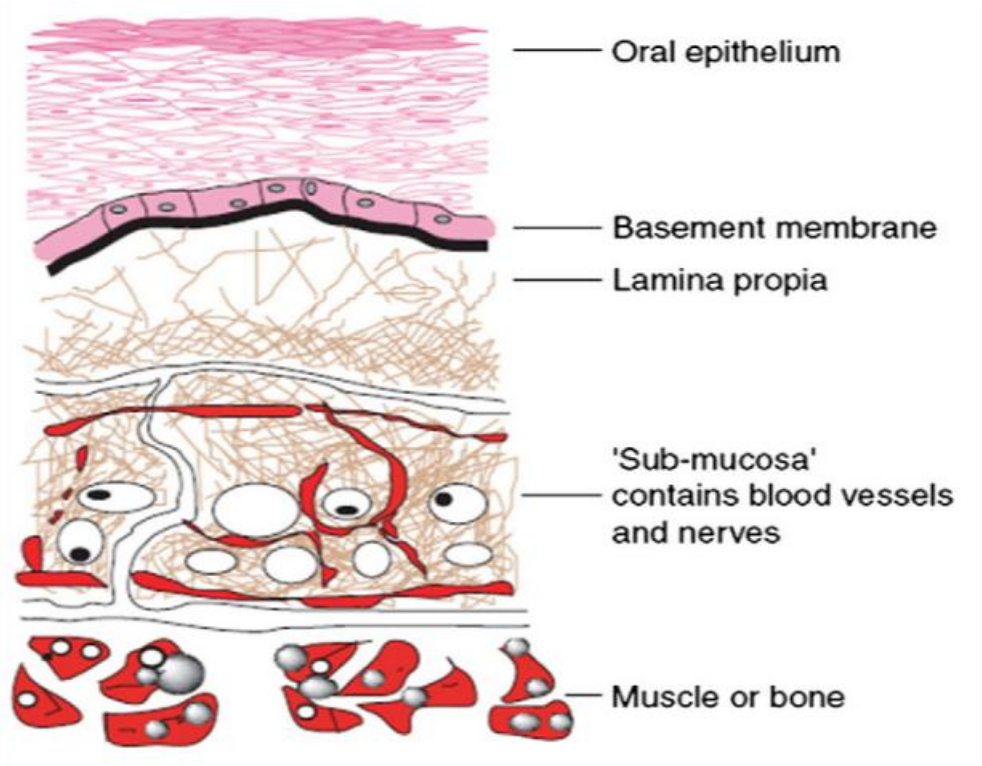
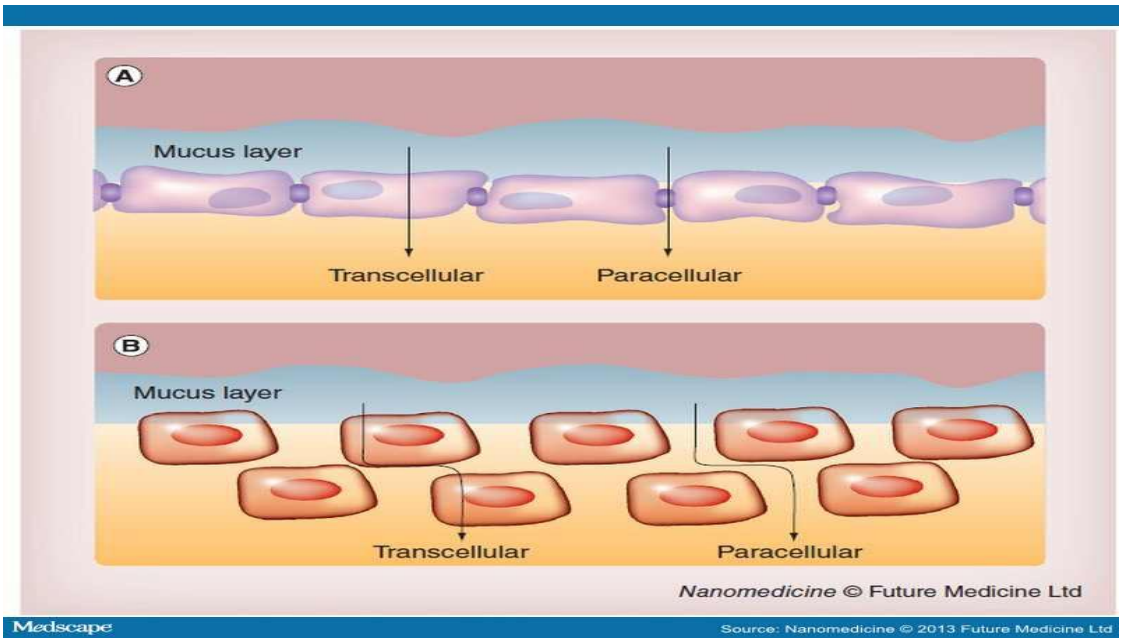


Fig. buccal mucosa.

Mechanism of drug transportation through buccal mucosa[7]

Transcellular or intracellular which demands to cross the cellular membrane with lipid and polar domain, but paracellular or intercellular transport is accomplished through passive diffusion through extracellular lipid domain.



Theories of Mucoadhesion[8–10]

Electronic Theory

This theory indicates that the electron transfer on contact with Bioadhesive polymer and glycoprotein network which have different electronic structure which will lead to formation of double layer of electronic charge at bioadhesive interface. The electrostatic attraction is occurs between two opposite charge polyelectrolyte.

Absorption Theory

The adsorption theory considers that the attraction between mucus and mucoadhesive polymer is achieved via specific forces like Vander wall, hydrogen bond. Hydrophobic effect may play important role when the mucoadhesive polymer is ampiphilic in nature.

Wetting Theory

This theory is applicable to liquid bio adhesive system and analyzes adhesion and contact behaviors in term of liquid or paste spread over biological surface. Dupres equation for work of adhesion is given by

$$w_A = \gamma_A + \gamma_B - \gamma_{AB}$$

Where A, and B are the biological membrane and bioadhesive formulation respectively.

This theory correlates the surface tension of the mucus and the mucoadhesive polymer with its ability to spread on the mucus layer.

Diffusion Theory

Mucoadhesive macromolecule into the mucus gel and diffuse of soluble mucin in dosage form resulting in formation of interpenetration layer. The diffusion theory considers the penetration rate being dependent upon the diffusion coefficients of both inter- acting polymers. The associate contact of two piece of same polymer or two different polymers during chain interpenetration of the molecule of the polymer and hanging chain of the glycoprotein are associated due to concentration gradient the bioadhesive polymer chain enter at the rates which are depends on different coefficient of macromolecule through a cross linked network and the chemical potential gradient. The bioadhesive polymer chain enter at the rate which are depends on different coefficient of macromolecule through the cross linked network.

Fraction Theory

This theory involves the force for polymer detachment from the mucus to the strength of their adhesive bond. Fraction theory allows the determination of fracture strength (σ) following the separation of two surfaces after adhesion to the adhesive bond strength via its relationship to Young's modulus of elasticity (E), the fracture energy (e) and the critical crack length (L) by the following equation:

$\sigma=(E \times e \div L)$

Cohesion Theory

This theory explains the Mucoadhesion occurs due to intermolecular interaction between the like molecules.

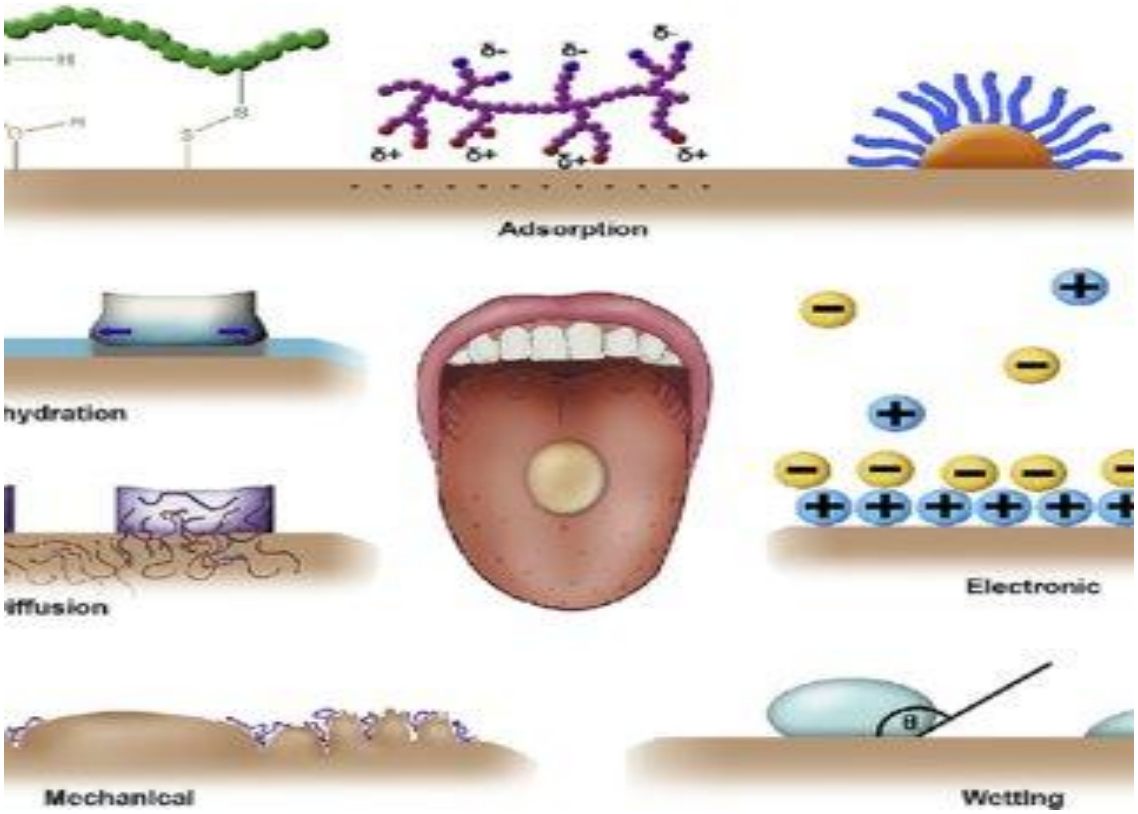


Fig. six mechanism of Mucoadhesion.

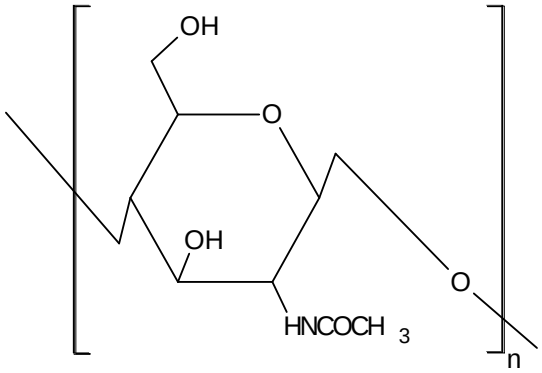
Various mucoadhesive dosage form including tablets[11], patches, wafers[12], nanoparticles[13], ointments and gels have been developed.[14] Amongst them mucoadhesive buccal films or patches is currently more prevalent for treatment due to its flexibility, comfort and greater absorption because of its large surface area and prolong residence time. Buccal film bypasses the drug from the hepatic first pass metabolism leading to high bioavailability[6,15] because of

direct access to the systemic circulation through the internal jugular vein. The drug release pattern of drug from film or patch is depending on the type of the polymers and design of system.[16] By means of avoiding hepatic first pass metabolism and enzyme or acid degradation in stomach and small intestine, the oral mucosa particularly buccal route is an alternative choice to deliver drugs to the application site. In addition this route also shows high patient compliance, a lower frequency of administration.[17] An ideal buccal delivery system should stay in the oral cavity for hours and release the drug in a controlled way for therapeutic action. Mucoadhesive polymers such as carbopol, polycarbophil, chitosan etc., prolong the residence time of the drug in the oral cavity. Chitosan is a promising polymer to be used for buccal delivery because of its mucoadhesive as well as absorption enhancement properties. In another study, the potential of thiolated chitosan for various drug delivery systems via buccal mucosa was investigated.[2]

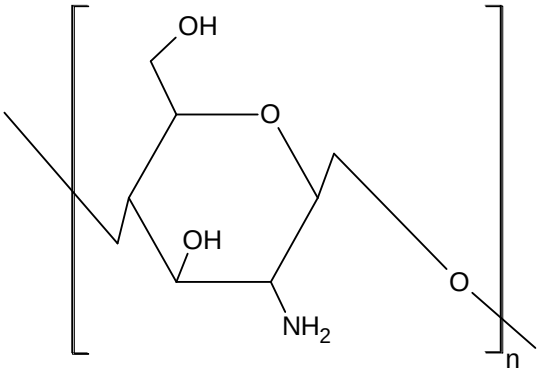
Chitosan and its Thiolated derivatives

Chitosan is the second most abundant biopolymer in nature after cellulose, is composed of β-(1−4)-linked D-glucosamine and N-acetyl-D-glucosamine units.[18] It is obtained by partial deacetylation of chitin which is the major component of the exoskeleton of crustaceans and the cell walls of fungi. Nowadays, chitosan are heavily applied in pharmaceutical and biomedical fields because of its availability, mucoadhesivity, and other favorable biological properties such as biodegradability, biocompatibility, and low immunogenicity.[19]

However, its limited mucoadhesive property and limited water-solubility at neutral and basic pH are considered as two major drawbacks of its use. Chemical modification of chitosan has been exploited to overcome these drawbacks, because it has reactive amino and hydroxyl groups. Various chemical modifications, including quaternization, thiolation, carboxylation, alkylation, acylation, PEGylation and graft copolymerization, which improve the beneficial properties of chitosan such as its aqueous solubility, mucoadhesivity as well as enzymatic inhibitory and tight junction opening abilities for oral drug delivery as compared to unmodified chitosan.[20] One of these modification ,the derivatization of the primary amino groups of chitosan with thiol group bearing reagents leads to the formation of thiolated chitosan's display, besides their strong mucoadhesivity, permeation enhancing effect, excellent cohesive properties as well as in-situ gelling properties.[21]



Chitin



Chitosan

Thiolated polymers are hydrophilic macromolecules exhibiting free thiol groups on the polymeric backbone also called as thiomers.[22] Due to these functional groups, various properties of well-established mucoadhesive excipients chitosan are strongly improved, via the formation of covalent disulfide bonds between thiol groups of the polymer and cysteine-rich sub-domains of mucus glycoproteins.[23] New generation of thiolated chitosan have two mechanisms: (1) improved ionic interactions between the strengthened cationic groups of modified chitosan and the anionic moieties provided by sialic acid and sulfonic acid of the mucus layer; and (2) the formation of disulfide bonds due to the introduction of thiol groups by reaction of chitosan with various thiol bearing regents[24] (as shown in table 1).

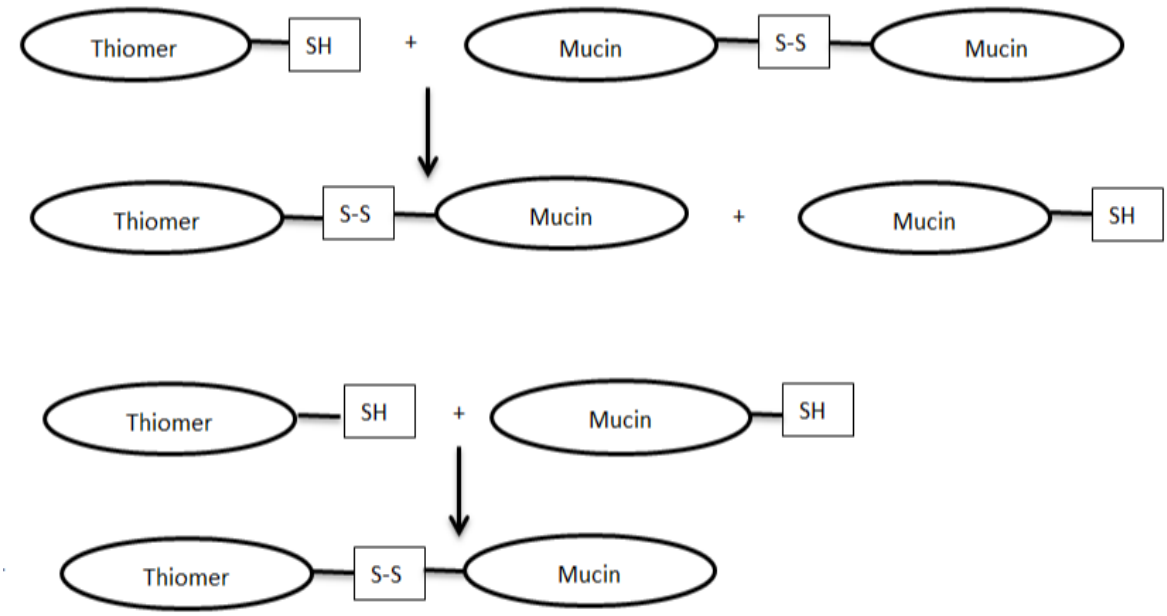


Fig. mechanism of disulfide bond formation between thiomers and mucus glycoproteins (mucin)

In the case of chitosan thioalkylamidine such as chitosan-4thiobutylamidine (TBA) and chitosan- thioethylamidine conjugates exhibit additionally increased mucoadhesive properties due to improved ionic interaction between cationic amidine substructure of the conjugates and anionic substructure of the mucus layer.

Table 1: Thiolated chitosan derivatives.

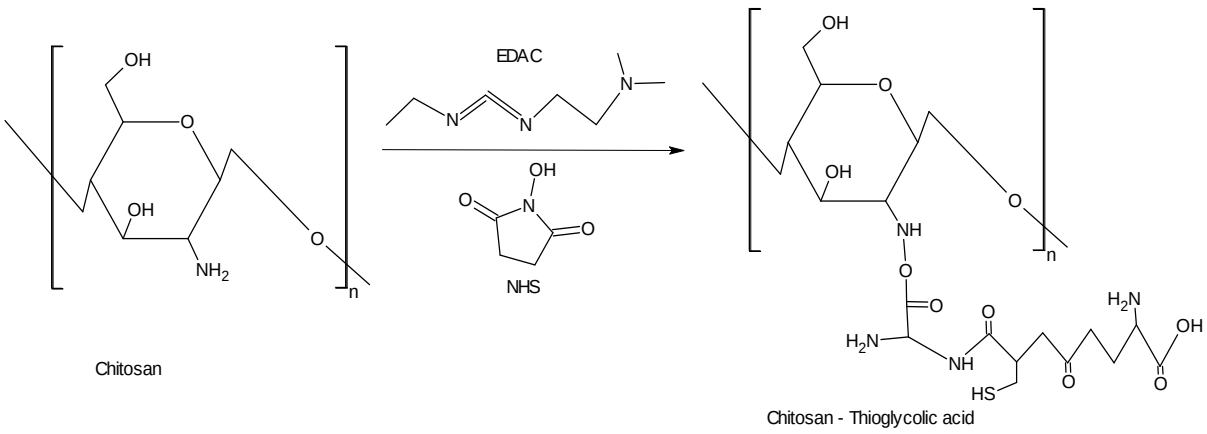
Sr no.	Chitosan derivatives	Thiolating agent	Properties	References
1	Chitosan thioglycolic acid (TGA)	Thioglycolic acid	Mucoadhesion, permeation enhancement and efflux inhibition	[25]
2	Chitosan- 4-thiobutylamidine (TBA)	2-iminothiolane	In situ gelling, mucoadhesive, provides cationic amidine substructure unlike TGA	[26]
3	Chitosan-thioethylamidine (TEA)	Isopropyl-*S*-acetylthioacetimidate	In situ gelling, mucoadhesive and to overcome insufficient stability.	[21-27]
4	Chitosan-6-mercaptonicotinic acid	6- mercaptonicotinic acid	Mucoadhesive, enzyme inhibitory and permeation enhancing properties, pH independent reactivity	[28]
5	Chitosan-3-mercaptopropionic acid	3-mercatopropionic acid	Mucoadhesion, permeation enhancement, cohesive property	[29]
6	Chitosan- glutathione	Glutathione	Mucoadhesion, permeation enhancement, in situ gelling.	[30]
7	Chitosan mercaptobenzoic acid	Mercaptobenzoic acid	Mucoadhesion, in situ gelling.	[31]

Synthesis of thiolated chitosan

The primary amino group at the second- position of the glucosamine subunits of chitosan is the main target for the immobilization of thiol groups, which are covalently linked to the primary amino group via the formation of amide or amidine bonds.[27] Besides all advantages of thiomers, a drawback of thiomers is their instability toward oxidation and pH-dependent reactivity.[32] Alternately, an unintended oxidation followed by disulfide bond formation during synthesis could be avoided by performing the reaction under inert condition.[33]

The thiol moiety bearing agents like cysteine, thioglycolic acid and glutathione[34] can be covalently via the amide bond formation between the primary amino group of chitosan and activated carboxylic acid group of the agent mediated by a water soluble condensing agent.

The carboxylic acid moieties of thiol containing reagent were activated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), forming an O-acyl urea derivatives as intermediate product, which reacts with the primary amino group of chitosan. For thiolation, EDC was added to 0.1% w/v acidic solution of chitosan in a final conc. of 50-125 mM, followed by addition of acid (glutathione or cysteine) in the ratio of 1:1 of polymer to acid. The reaction mixture was incubated at pH 5 for 3 hrs at RT with continuous stirring. The thiolated product was purified by dialysis: once against 5 mM HCl: twice against same medium, but containing 1% NaCl, to reduce ionic interaction between cationic polymer and the anionic sulfhydryl compound and twice against one mili molar HCl to adjust the pH of the polymer to 4. The lyophilisation of aqueous polymer solution at -30° temp., and 0.01 mbar gave the thiomers in powder form. The extent of thiolation was pH dependent for glutathione between pH 5-6 and was optimum at pH 5.5. At this pH range, the conc. of thiolated anions, demonstrating reacting form for oxidation of thiol groups, was low, and the formation of disulphide bond could be almost excluded. Alternately, an unintended oxidation of thiol groups during synthesis could be avoided by performing the reaction under inert condition. The reaction conditions were adopted for modification with acetyl cysteine and reduced form glutathione employed in large quantities as 1-2 gm for 0.5-1 gm of chitosan.[32]



Chitosan - Thioglycolic acid

Fig. synthetic pathway for the generation of Chitosan- Thioglycolic acid conjugate.

The modifying reagent for chitosan- 4- thiobutylamidine conjugate is 2- iminothiolane or Traut's reagent, which reacts with amino groups of chitosan and introduces a sulfhydryl residue via a positively charged amidine substructure. However, storage stability studies under nitrogen showed an inadequate stability of thiomers, which results in a decrease of free thiol groups. This may be due to the formation of N-chitosanyl-substituted 2-iminithiolane structures. This undesired side reaction occurs after derivatization of different amines with 2-

iminothiolane. It involves the loss of ammonia and yields recyclized N-substituted 2-iminothiolane.[21] To avoid an oxidation during coupling reaction, optionally 2-mercaptoethanol was added in a final concentration of 3% (v/v).[33-35]

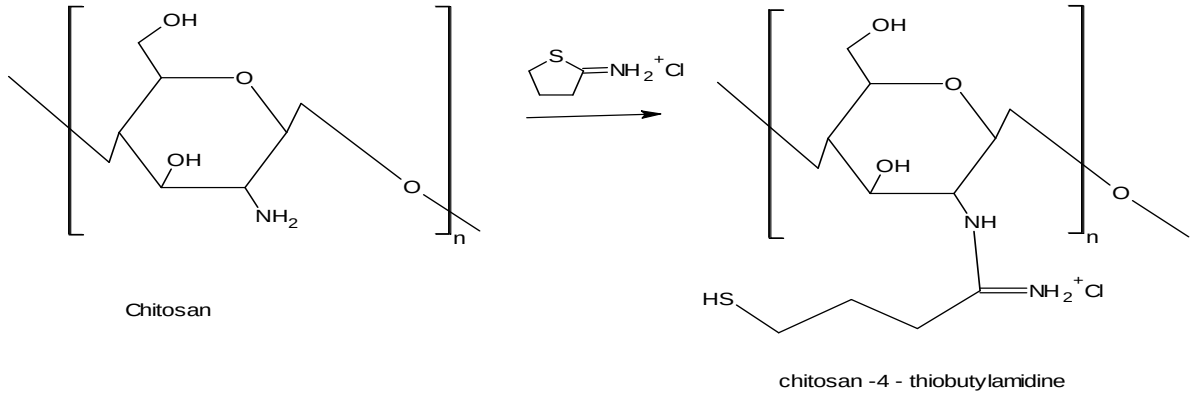


Fig. synthetic pathway for modification of chitosan with 2-iminothiolane.

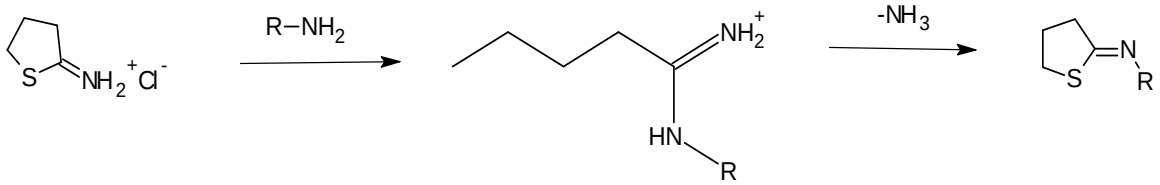


Fig. Formation of an N-substituted 2-iminothiolane.

In order to achieve the same properties as chitosan-4-thiobutylamidine and to guarantee, on the other hand, also the stability of this novel excipient was achieved by the modification of chitosan with the new reagent: isopropyl-*S*-acetylthioacetimidate (*i*-PATAI.HCl), bearing a protected thiol moiety.[21,27]

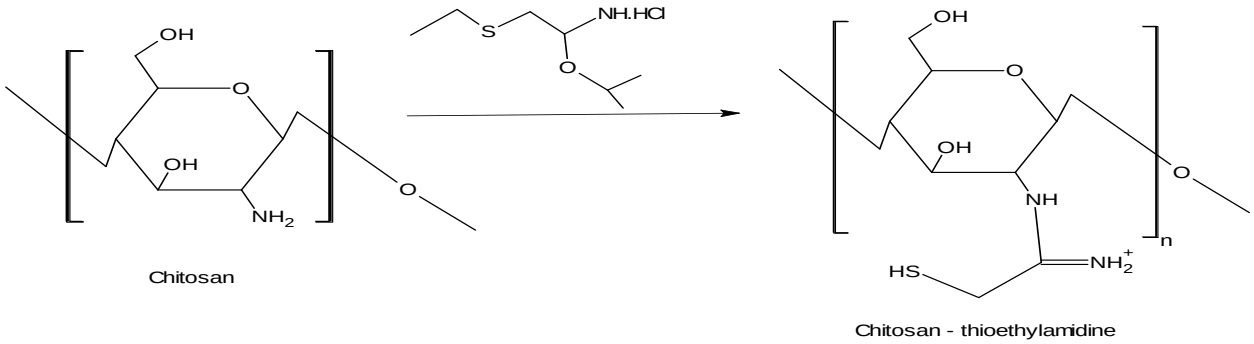


Fig. Synthetic pathway of chitosan- TEA conjugates.

Thiolated chitosan composed Mucoadhesive film

The usefulness of thiolated chitosan as carrier matrices for controlled drug release was demonstrated with model drug such as, clotrimazole[36–38], fluconazole[39], chlorhexidine gluconate[4], atenolol.[40] Basic component of buccal film are drug, bioadhesive polymer,

backing layer, permeation enhancer, plasticizer. The selection of drug for the design of mucoadhesive drug delivery systems should be based on pharmacokinetic properties. Bioadhesive films are usually prepared by a solvent casting method and hot extrusion technique.[6] The solvent casting method is most commonly used technique for film formation. In solvent casting method, water soluble ingredients such as polymers and plasticizer are dissolved in water to form homogenous viscous solution. Then, drug is dissolved in suitable solvent. Both the solutions are mixed and stirred it. Keep aside for 5-6 hrs for degassing and resulting solution is then cast into films followed by drying and finally laminated with a backing layer or a release liner, which retard the diffusion of saliva into drug layer, thus increases the adhesion time and reducing the drug loss.[2]

Kiran Naz and co-workers carried out formulation and in-vitro evaluation of thiolated buccoadhesive film of Fluconazole.[39] The investigation highlights the unmodified polymers chitosan backbone was covalently modified by thioglycolic acid (TGA) and thiolated chitosan was employed for synthesis of film.

Evaluation parameters for Film[39,41]

Tensile strength studies

Tensile strength measures the mechanical properties of the film which affect the Mucoadhesion and calculated by dividing the load at break by the original minimum cross sectional area. The result is expressed in mega Pascal (MPa) and reported to three significant figures.

$$\text{strenght} = \frac{\text{load at break}}{\text{original widthtensile} \times \text{original lenght}} \times 100$$

Fig. Thiolated chitosan derivatives.

Determination of drug entrapment

Drug entrapment of the buccal film and thiolated mucoadhesive film was calculated by dissolving the film in 5 mL of 0.1 N acetic acid and stirred continuously for 6 h for complete drug extraction. From this solution, 0.2 mL of solution was transferred to the beaker and final volume was made up to 10 mL with acetic acid. Absorbance was measured using UV- visible spectrophotometer at 261 nm, and the readings were recorded. The entrapment of the drug was calculated by the formula:

$$\text{Drug Entrapment} = \frac{\text{amount of drug present in film}}{\text{amount of drug adeed in formulation}} \times 100$$

Thickness of the film

The thickness of film measured by micrometer gauge or calibrated digital Vernier Callipers. The thickness of film must be in range 5-200µm. The thickness should be evaluated at five different points (four corners and one center). To ascertain uniformity in the thickness of film is directly related to accuracy of dose distribution in the film.

CONCLUSION

In this review, general methods of synthesis of potential mucoadhesive thiolated chitosan derivatives have been highlighted. To overcome the obstacles in oral cavity drug delivery such as weak adhesion on mucosal surfaces, swallowing of materials and wash out effect, in-situ crosslinking and curing of Mucoadhesion polymeric film are required. The mucoadhesive properties of thiolated chitosan derivatives have been particularly considered in this article. Thiolated chitosan derivative are multifunctional polymers that exhibit improved mucoadhesive, cohesive and permeation-enhancing as well as efflux-pump- inhibitory properties. They can be synthesized by chemical modification of the primary amino groups of chitosan with coupling reagents bearing thiol functions. Due to their high mucoadhesivity and unique physiological feature thiolated chitosan has great interest in controlled drug delivery. Hence this article focuses on the novel mucoadhesive buccal film and thiolated chitosan.

REFERENCES

1. Guo Y, Singh AP. Journal of Drug Delivery Science and Technology Emerging strategies for enhancing buccal and sublingual administration of nutraceuticals and pharamaceuticals. *J Drug Deliv Sci Technol*, 2019; 52(April): 440-451. doi:10.1016/j.jddst.2019.05.014
2. Salamat-miller N, Chittchang M, Johnston TP. The use of mucoadhesive polymers in buccal drug delivery B., 2005; 57: 1666-1691. doi:10.1016/j.addr.2005.07.003
3. Patel VF, Liu F, Brown MB. Advances in oral transmucosal drug delivery. *J Control Release*, 2011; 153(2): 106-116. doi:10.1016/j.jconrel.2011.01.027
4. Kas S, Sargon MF. Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal delivery, 2000; 193: 197-203.
5. Chinna Reddy P, Chaitanya KSC, Madhusudan Rao Y. A review on bioadhesive buccal drug delivery systems: Current status of formulation and evaluation methods. *DARU, J Pharm Sci*., 2011.
6. Madhavi B R. Buccal Film Drug Delivery System-An Innovative and Emerging

Technology. *J Mol Pharm Org Process Res.*, 2016; 1(3). doi:10.4172/2329-9053.1000107

7. Laffleur F, Bernkop-Schnürch A. Strategies for improving mucosal drug delivery. *Nanomedicine*, 2013. doi:10.2217/nnm.13.178
8. Manohar SD, Sridhar DA, Mallikarjuna SC. Drug delivery from the oral cavity: A focus on mucoadhesive buccal drug delivery systems. *PDA J Pharm Sci Technol*, 2012. doi:10.5731/pdajpst.2012.00877
9. Gandhi RB, Robinson JR. Oral cavity as a site for bioadhesive drug delivery. *Adv Drug Deliv Rev.*, 1994. doi:10.1016/0169-409X(94)90026-4
10. Cook SL, Bull SP, Methven L, Parker JK, Khutoryanskiy V V. Mucoadhesion: A food perspective. *Food Hydrocoll*, 2017; 72(June): 281-296. doi:10.1016/j.foodhyd.2017.05.043
11. Singh S, Jain S, Muthu M, Tilak R. Preparation and Evaluation of Buccal Bioadhesive Tablets Containing Clotrimazole. *Curr Drug Deliv*, 2008. doi:10.2174/156720108783954879
12. Boateng JS, Ayensu I. Preparation and characterization of laminated thiolated chitosan-based freeze-dried wafers for potential buccal delivery of macromolecules. *Drug Dev Ind Pharm*, 2014. doi:10.3109/03639045.2014.884126
13. Suh JW, Lee JS, Ko S, Lee HG. Preparation and Characterization of Mucoadhesive Buccal Nanoparticles Using Chitosan and Dextran Sulfate. *J Agric Food Chem.*, 2016; 64(26): 5384-5388. doi:10.1021/acs.jafc.6b00849
14. Paderni C, Compilato D, Giannola LI, Campisi G. Oral local drug delivery and new perspectives in oral drug formulation. *Oral Surg Oral Med Oral Pathol Oral Radiol*, 2012. doi:10.1016/j.oooo.2012.02.016
15. Juliano C, Cossu M, Pigozzi P, Rassu G, Giunchedi P. Preparation, in vitro characterization and preliminary in vivo evaluation of buccal polymeric films containing chlorhexidine. *AAPS PharmSciTech.*, 2008. doi:10.1208/s12249-008-9153-6
16. Shirvan AR, Bashari A, Hemmatinejad N. New insight into the fabrication of smart mucoadhesive buccal patches as a novel controlled-drug delivery system. *Eur Polym J.*, 2019; 119(June): 541-550. doi:10.1016/j.eurpolymj.2019.07.010
17. Ciro Y, Rojas J, Salamanaca CH. Thiolated Chitosan: A Promising Strategy for Improving the Effectiveness of Anticancer Drugs. *B Chithin Proporties, Apl Res.*, 2017; (Cv): 1-17.
18. Azuma K, Izumi R, Osaki T, et al. Chitin, Chitosan, and Its Derivatives for Wound

Healing: Old and New Materials. *J Funct Biomater*, 2015; 6(1): 104-142. doi:10.3390/jfb6010104

19. Mahanta AK, Maiti P. Injectable Hydrogel through Hydrophobic Grafting on Chitosan for Controlled Drug Delivery, 2019. doi:10.1021/acsabm.9b00733
20. Chen M, Mi F, Liao Z, et al. Recent advances in chitosan-based nanoparticles for oral delivery of macromolecules ☆. *Adv Drug Deliv Rev*., 2012. doi:10.1016/j.addr.2012.10.010
21. Kafedjiiski K, Krauland AH, Hoffer MH, Bernkop-Schnürch A. Synthesis and in vitro evaluation of a novel thiolated chitosan. *Biomaterials*, 2005; 26(7): 819-826. doi:10.1016/j.biomaterials.2004.03.011
22. Bonengel S, Bernkop-Schnürch A. Thiomers - From bench to market. *J Control Release*, 2014; 195: 120-129. doi:10.1016/j.jconrel.2014.06.047
23. Leitner VM, Walker GF, Bernkop-schnu A. Thiolated polymers : evidence for the formation of disulphide bonds with mucus glycoproteins, 2003; 56: 207-214. doi:10.1016/S0939-6411(03)00061-4
24. Roldo M, Hornof M, Caliceti P, Bernkop-Schnürch A. Mucoadhesive thiolated chitosans as platforms for oral controlled drug delivery: Synthesis and in vitro evaluation. *Eur J Pharm Biopharm*, 2004; 57(1): 115-121. doi:10.1016/S0939-6411(03)00157-7
25. Kast CE, Bernkop-schnu A. Thiolated polymers * thiomers : development and in vitro evaluation of chitosan} thioglycolic acid conjugates, 2001; 22.
26. Bernkop-Schnürch A, Hornof M, Zoidl T. Thiolated polymers - Thiomers: Synthesis and in vitro evaluation of chitosan-2-iminothiolane conjugates. *Int J Pharm*, 2003. doi:10.1016/S0378-5173(03)00271-0
27. Kafedjiiski K, Hoffer M, Werle M, Bernkop-Schnürch A. Improved synthesis and in vitro characterization of chitosan- thioethylamidine conjugate. *Biomaterials*, 2006; 27(1): 127-135. doi:10.1016/j.biomaterials.2005.05.075
28. Millotti G, Samberger C, Fro E, Bernkop-schnu A. Chitosan- graft -6-mercaptonicotinic Acid : Synthesis, Characterization, and Biocompatibility, 2009: 3023-3027.
29. Esquivel R, Juárez J, Almada M, Ibarra J, Valdez MA. Synthesis and characterization of new thiolated chitosan nanoparticles obtained by ionic gelation method. *Int J Polym Sci*., 2015; 2015. doi:10.1155/2015/502058
30. Kafedjiiski K, Föger F, Werle M, Bernkop-Schnürch A. Synthesis and in vitro evaluation of a novel chitosan-glutathione conjugate. *Pharm Res*., 2005. doi:10.1007/s11095-005-

6248-6

31. Millotti G, Samberger C, Fröhlich E, Sakloetsakun D, Bernkop-Schnürch A. Chitosan-4-mercaptobenzoic acid: Synthesis and characterization of a novel thiolated chitosan. *J Mater Chem*., 2010. doi:10.1039/b916528b
32. Dünnhaupt S, Barthelmes J, Rahmat D, et al. S-protected thiolated chitosan for oral delivery of hydrophilic macromolecules: Evaluation of permeation enhancing and efflux pump inhibitory properties. *Mol Pharm*., 2012; 9(5): 1331-1341. doi:10.1021/mp200598j
33. Inamdar NN, Mourya V. Thiolated chitosan preparation, properties, and applications. In: *Chitin and Chitosan Derivatives: Advances in Drug Discovery and Developments*, 2013. doi:10.1201/b15636
34. Koo SH, Lee JS, Kim GH, Lee HG. Preparation, characteristics, and stability of glutathione-loaded nanoparticles. *J Agric Food Chem*., 2011. doi:10.1021/jf2024648
35. Bernkop-schnu A, Guggi D, Pinter Y. Thiolated chitosans : development and in vitro evaluation of a mucoadhesive, permeation enhancing oral drug delivery system, 2004; 94: 177-186. doi:10.1016/j.jconrel.2003.10.005
36. Khanna R, Agarwal SP, Ahuja A. *Preparation and Evaluation of Muco-Adhesive Buccal Films of Clotrimazole for Oral Candida Infections*. Vol 59.; 1997.
37. Wang LF, Rhim JW, Hong SI. Preparation of poly(lactide)/poly(butylene adipate-co-terephthalate) blend films using a solvent casting method and their food packaging application. *LWT - Food Sci Technol*, 2016. doi:10.1016/j.lwt.2015.12.062
38. Singh S, Jain S, Muthu MS, Tiwari S, Tilak R. Preparation and evaluation of buccal bioadhesive films containing clotrimazole. *AAPS Pharm Sci Tech*., 2008. doi:10.1208/s12249-008-9083-3
39. Naz K, Shahnaz G, Ahmed N, et al. Formulation and In Vitro Characterization of Thiolated Buccoadhesive Film of Fluconazole. *AAPS Pharm Sci Tech*., 2017; 18(4): 1043-1055. doi:10.1208/s12249-016-0607-y
40. Adhikari SNR, Nayak BS, Nayak AK, Mohanty B. Formulation and Evaluation of Buccal Patches for Delivery of Atenolol, 2010; 11(3): 1038-1044. doi:10.1208/s12249-010-9459-z
41. Nair AB, Kumria R, Harsha S, Attimarad M, Al-Dhubiab BE, Alhaider IA. In vitro techniques to evaluate buccal films. *J Control Release*, 2013. doi:10.1016/j.jconrel.2012.11.019