

## Chitosan: A Novel Functional Excipient Used in Mucoadhesive Formulation

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### ABSTRACT

Polymers have been used as a main tool to control the drug release rate from the formulations. Extensive applications of polymers in drug delivery have been realized because polymers offer unique properties which so far have not been attained by any other materials. Chitosan is a cationic natural polysaccharide which is derived from the chitin of crustaceans, with crabs and shrimp-shell wastes as its principal source. Its properties include extent of deacetylation and the average molecular weight of polymer as well as low toxicity and good bioavailability make it a novel excipient in pharmaceutical formulation as a relatively new development. Together with chitin, chitosan is well thought-out the second most abundant polysaccharide subsequent to cellulose. But in contrast to cellulose, the application of Chitosan in pharmaceutical field is a pretty new development. Recently there are so many mucoadhesive formulation were prepared and evaluated within different dosage forms such as ophthalmic, nasal sublingual, buccal, periodontal, gastrointestinal, colon specific, vaginal, transdermal as well as gene carrier which is based on the application of chitosan and its derivatives. Chitosan is biocompatible and show the activities such as antimicrobial and antifungal activities, which makes it a favourable option for biomedical applications. It has been proven to be useful in tissue growth, in tissue repair and accelerating wound-healing and bone regeneration. Microcrystalline chitosan (MCCh) is a highly crystalline grade of chitosan base may be particularly valuable as an excipient. Mucoadhesive tendency of chitosan might also depend on its crystallinity. Efficient gel formation by MCCh could result in substantial mucoadhesion, at least as far as "adhesion by hydration" is concerned. The objective of this review is to summarize the application and formulation based on the chitosan and its derivatives and also to elaborate the importance of chitosan in pharmaceutical field.

**Keywords:** Chitosan, natural polymers, excipient, drug delivery systems

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### INTRODUCTION

Polymers have been used as a main tool to control the drug release rate from the formulations. Among the novel families of biological macromolecules, whose relevance is becoming increasingly evident, are chitin and its main derivative, chitosan. Potential and usual applications of chitin, chitosan and their derivatives are estimated to be more than 200. This wide range of applications includes biomedicine, food, biotechnology, agriculture and cosmetics, among others.

Chitosan is a hydrolyzed (deacetylated) derivative of chitin, a biopolymer widely distributed in nature and biologically safe (1,2). This polymer exhibits several

favorable properties, such as biodegradability and biocompatibility. It also has mucoadhesive properties due to its positive charges at neutral pH that enable an ionic interaction with the negative charges of sialic acid residues of the mucus (3,4). Some of which include binding, disintegrating, and tablet coating properties. Numerous studies have demonstrated that chitosan and its derivatives (N-trimethyl chitosan, mono-N-carboxymethyl chitosan) are effective and safe absorption enhancers to improve mucosal (nasal, peroral) delivery of hydrophilic macromolecules, such as peptides, proteins, and heparins.

Chitosan is a cationic polymer derived from the chitin of crustaceans. Its use in pharmaceutical field has received considerable attention, because it can be obtained from ecologically sound natural sources, namely crab- and shrimp-shell wastes. However Chitosan has been widely studied in the biomedical field, and has been found to be highly biocompatible 1. Together with chitin, Chitosan is second most abundant polysaccharide subsequent to cellulose. However contrasting cellulose, the employ of Chitosan as an excipient in pharmaceutical formula is a pretty new development.

Now chitosan is available in different grades having difference in their physicochemical properties, and as a base or a salt of a base. This existence of different grades could also be valuable. The properties of chitosan-based dosage forms could be controlled by altering the grade of chitosan in formulations. Chitosan base seems likely to be more useful than chitosan salts in relation to development of slow-release formulations because it is in general less soluble than Chitosan salts. The Chitosan is differs from chitin in that a majority of the N-acetyl groups in chitosan are hydrolyzed. The degree of hydrolysis (deacetylation) has a significant effect on the solubility and rheological properties of the polymer. The amine group on the polymer has a pKa in the range of 5.5 to 6.5, depending on the source of the polymer (2).

From a biopharmaceutical point of view, chitosan act as both the mucoadhesive as well as permeability enhancing property across the epithelia. It has been proved that chitosan could enhance insulin absorption across human intestinal epithelial cells without injuring them (3, 4, 5).

Microcrystalline chitosan (MCCh) is a highly crystalline grade of chitosan base(6), may be particularly valuable as an excipient, which can be prepared on a large scale using a method developed by the Finnish company Novasso (7). MCCh has been studied in relation to various technical (8 ) and in cholesterol- lowering formulations (9). Most drug formulation studies involving chitosans have used material produced commercially by conventional methods.

Chitosan of this kind are fairly amorphous. However, it was reported that increasing the crystallinity of chitosan could offer advantages in relation to manufacturing process of pharmaceutical formulations, e.g. by making the chitosan more suitable for direct compression into tablets (10). Perhaps more significantly, the crystallinity of a chitosan could affect the behaviour of a formulation in which it was incorporated. Effects of the crystallinity of chitosan therefore required evaluation. One specific property of MCCh is its high capacity for retaining water (6).

Another advantage of Chitosan as pharmaceutical excipient is that it opens the tight junction of the mucosal barrier and facilitates the paracellular transport of hydrophilic macromolecules (11).

Due to mucoadhesive properties of chitosan drug strongly adheres to mucosa and MCC is decreased thus increasing the residence time of drug in nasal cavity which results in increase in absorption (12, 13). It has been claimed that chitosan entraps lipids in the intestine, because of its cationic nature (14, 15). Chitosan may also have technical applications, including, e.g., use as seed coating and nitrogen source in agriculture, and as an adsorbent for water-purification (16).

#### **Origin, Chemistry and Derivatives of Chitosan:**

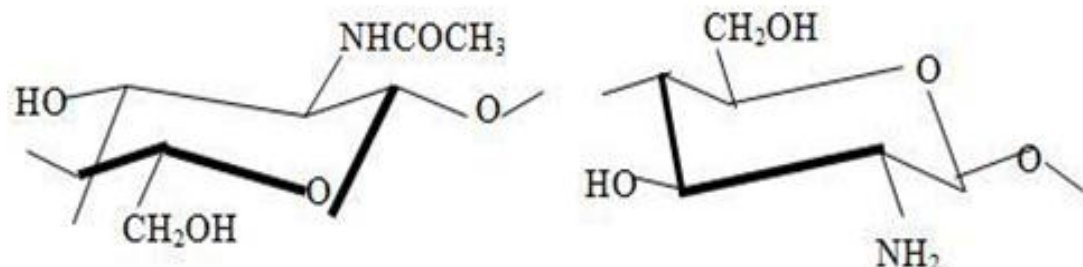
Origin: Henri Bracannot, Director of the Botanical Garden in Nancy, France, who firstly discovered Chitosan in 1811, pragmatic that a definite substance (chitin) set up in mushrooms did not dissolve in sulphuric acid. It was till 20 years regarding Chitosan after that, there was a man who identified that amazing substance was present in the structure of insects as well as the structure of plants, called as "chitin" which is derived from Greek, connotation "tunic" or "envelope".

Chitin is the primary structure component of the outer skeleton of the crustaceans, molluscs, insects and fungi. While experimenting with chitin, Rouget first discovered Chitosan in which He observed that by the manipulation in the compound of chitin through chemical and temperature treatments it become soluble. Several other researchers continue to build on the

original finding of Bracannot, discovering new uses for chitin as they find different forms of it in nature. In 1878, Ledderhose described chitin that it is made of glucosamine and acetic acid (17, 18). Chitin accounts for approximately 70% of the organic components in such shells. It is a

reinforcing material, which occurs in three polymorphic forms,  $\alpha$ -,  $\beta$ - and  $\gamma$ -chitin.

When chitin is heated in a strong solution of sodium hydrochloride (>40%) at high temperature (90-120°C), mostly of N-acetyl-D-glucosamine-units (left) of chitin, are deacetylated to D-glucosamine -units (right).



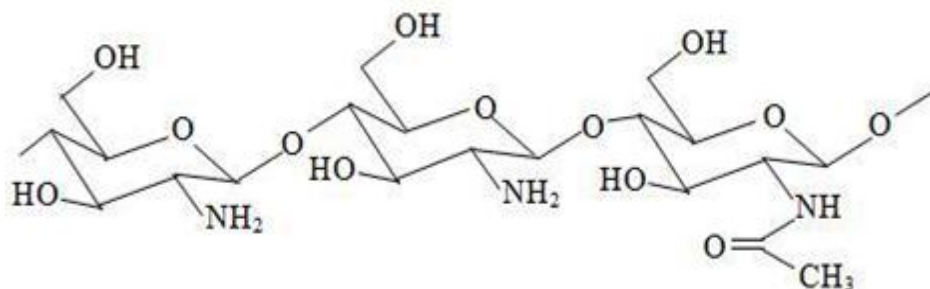
**Figure 1: Structural Units of Chitosan and its Parent Substance Chitin**  
Chitin consists mostly of N-acetyl-D-glucosamine-units (left). During the preparations of Chitosan, most units are deacetylated to D-glucosamine -units (right)

#### Chemistry:

Chitosan (Poly[-(1, 4)-2-amino-2-deoxy-D-glucopyranose]) has a structure as shown in figure - 2. Chitin is isolated from shells of crustacean (for example shrimp, crab and lobster) by treating the shells with 2.5 N NaOH at 75°C and with 1.7 N HCl at room temperature for 6 hours (19). Deacetylation can be done by alkaline treatment or by enzymatic reaction. The alkaline deacetylation is carried out by treating chitin with NaOH at high temperature. The degree of deacetylation increases with increasing temperature or NaOH concentration. Determined the optimum deacetylation is done by mixing 23 ml of 60% NaOH per gram of chitin 170°C (20). Chitin deacetylation by enzymatic reaction is described. Chitin deacetylase isolated from *Mucor rouxii* has been used

successfully to deacetylate chitin almost completely (98%) (21).

The polymer differs from chitin in that a majority of the N-acetyl groups in Chitosan is hydrolyzed. The degree of hydrolysis has a significant effect on the solubility and rheological properties of the polymer. The amino group on the polymer has a pKa in the range of 5.5 to 6.5, depending on the source of the polymer. At low pH, the polymer is soluble, with the sol-gel transition occurring at approximate pH 7. The pH sensitivity coupled with the reactivity of the primary amino groups makes chitosan a unique polymer for and drug delivery applications. Chitosan is now available commercially in various molecular weights (50 kDa – 2,000 kDa) and different degree of deacetylation (40% to 90%) (22).



**Figure 2: Chemical Structure of Chitosan**  
The polymer is obtained by the partial deacetylation of naturally occurring polymer, chitin

**Derivatives of chitosan:**

Chitosan provides a number of excellent properties, further derivatization of the amine functionalities can be carried out to obtain polymers with a range of properties. A number of approaches, both chemical and enzymatic, have been tried to exploit the reactivity of the amine functional groups 2.

**1. N-Trimethylene Chloride Chitosan (TMC):**

A number of studies demonstrated that the charge on chitosan has a role in providing intestinal permeability. Hence, a quaternary derivatized chitosan (N-trimethylene chloride chitosan) was shown to demonstrate higher intestinal permeability than chitosan alone. The TMC derivative was used as a permeation enhancer for large molecules, such as octreotide, a cyclic peptide. It was showed that the degree of quaternization of TMC influences its drug absorption-enhancing properties. Polymers with higher degrees of quaternization (> 22%) were able to reduce the transepithelial electrical resistance and thereby epithelial transport (in-vitro) in a neutral environment (pH 7.4). The maximum reduction in transepithelial resistance was reached with TMC with a degree of quaternization of 48%. This degree of quaternization was also seen to be optimum for in vitro transport of model drugs across a Caco-2 monolayer (23).

**2. Chitosan Esters:**

Chitosan esters, such as chitosan succinate and chitosan phthalate have been used successfully as potential matrices for the colon-specific oral delivery of sodium diclofenac (24). By converting the polymer from an amine to a succinate form, the solubility profile is changed significantly. The modified polymers were insoluble under acidic conditions and provided sustained release of the encapsulated agent under basic conditions. The same researchers also synthesized an iron cross-linked derivative of hydroxamated chitosan succinate, as a matrix for oral theophylline beads (25). A similar colon-targeting application was suggested for this polymer as well.

**3. Chitosan Conjugates:**

Reactivity of the amine functionality can be exploited to covalently conjugate functional

excipients to the polymer backbone. For example, Guggi and Bernkop attached an enzyme inhibitor to chitosan. The resulting polymer retained the mucoadhesivity of chitosan and further prevented drug degradation by inhibiting enzymes, such as trypsin and chymotrypsin (26). This conjugated chitosan demonstrated promise for delivery of sensitive peptide drugs, such as calcitonin.

**4. Water-soluble derivative of chitosan at neutral pH:**

Chitosan and its derivatives soluble in pH values of lower than 6.0 may not be desirable for usage in medicine, cosmetics and food (27). To improve its solubility at neutral pH, it is first derivatized with substituent's containing quaternary amino group (28), caboxymethylation and then sulfatation by adding strongly hydrophilic substituent (29).

**5. N- Sulfonated derivatives of Chitosan:**

These are amphoteric in nature. They can be prepared under heterogeneous reaction condition using 2-sulfobenzoic acid anhydride 30, N-sulfonato-N, O-carboxymethyl- chitosan: a novel polymeric absorption enhancer for the oral delivery of macromolecules (31).

**6. Quaternarized Derivatives:**

The simplest derivative is the trimethyl ammonium salt of chitosan. A repeated treatment of chitosan in N-methyl-2 pyrrolidone containing sodium iodide and methyl iodide with chloride ion in presence of sodium hydroxide results into the trimethyl ammonium salt of chitosan with high degree of substitution (32). Anionic changes of iodide with chloride ion are necessary for stabilization. The resulting product is water soluble at neutral pH (33).

**7. Carboxyalkylation:**

The process of carboxyalkylation introduces acidic group on the polymer backbone. This derivative exhibits amphotericity due to the presence of native amino group. Water solubility is attained at pH values above or below the isoelectric point. Formation of N-carboxyalkylation uses carboxyaldehyde in a reductive amination sequence (34). The reaction is carried out under homogeneous condition provided that the aldehyde used is water, allowing for greater degree of substitution distribution along the polymer

back-bone. However, sequential substitution gives rise to the formation of bis- carboxymethyl derivatives have been observed using glyoxalic acid (35).

#### **8. Microcrystalline Chitosan:**

MCCh differs from conventional chitosan in respect of greater crystallinity, energy of hydrogen bonds, and water retention. Both high energy of hydrogen bonds and high water retention are properties reflecting the increase in crystallinity and the substantial surface area of MCCh. The ability of MCCh to retain high amounts of water is a property which could be of particular value in relation to slow-release formulations. MCCh can retain three to four times much water as the parent chitosan (6). This might result in MCCh having a greater capacity than conventional chitosan to form gels in formulations, and result in marked retardant effects on drug release.

#### **Mucoadhesive drug delivery system**

Mucoadhesive drug delivery system may be defined as drug delivery system that utilize the property of bioadhesion of certain suitable polymer which become adhesive when used for targeting a drug to particular region of the body for extended period of time. Mucoadhesive polymers may provide an important tool to improve the bioavailability of the active agent by improving the residence time at the delivery site. The various sites where mucoadhesive polymers have played an important role include buccal cavity, nasal cavity, rectal lumen, vaginal lumen and gastrointestinal tract. Development of novel mucoadhesive delivery systems are being undertaken

so as to understand the various mechanism of mucoadhesion and improved permeation of active agents Mucoadhesive tendency of chitosan might also depend on its crystallinity. Efficient gel formation by MCCh could result in substantial mucoadhesion, at least as far as "adhesion by hydration" is concerned. Results of studies relating to technical applications of chitosan have indicated that the reactivity of MCCh is greater than that of conventional chitosan, because of the greater ability of MCCh to form hydrogen bonds (8). Because adhesion of chitosan to mucosa takes primarily through hydrogen bonding and

electrostatic interactions, differences in ability to form hydrogen bonds might be reflected in differences in capacity to adhere to mucosa.

Stability and storage condition: Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. It should be stored in cool, dry place; preferably at a temperature of 2-8°C. Chitosan is incompatible with strong oxidizing agent (36).

Chitosan has also recently been approved by the authorities, and a monograph relating to chitosan hydrochloride was included in the fourth edition of the European Pharmacopoeia (2002). It was confirmed that several properties of chitosan like good biocompatibility and low toxicity of chitosan and the fact that sources of chitosan are abundant, make it potentially valuable as a pharmaceutical excipient.

#### **Ideal characteristic:**

Characteristics of an ideal mucoadhesive polymers (37)

- The polymer and its degradation products should be nontoxic and should be nonabsorbable from the gastrointestinal tract.
- It should be non-irritant to the mucous membrane.
- It should preferably form a strong non-covalent bond with the mucin-epithelial cell surfaces.
- It should adhere quickly to most tissue and should possess some site-specificity.
- It should allow incorporation to the daily dose of the drug and offer no hindrance to its release.
- The polymer must not decompose on storage or during the shelf life of the dosage form.
- The cost of polymer should not be high so that the prepared dosage form remains competitive.

#### **Polymer related factors**

The adhesive bond between a bioadhesive system and mucin gel can be investigated in term of contribution of the following factors: (38)

- Molecular weight- With the increase in the molecular weight (MW) of the polymer chain there is an increase in the mucoadhesiveness of a polymer.

- Chain length- With the increase in the chain length of the polymers there is an increase in the mucoadhesive property of the polymer.
- Spatial arrangement- spatial conformation of a molecule is also important factor.
- Flexibility- Flexible polymer chains helps in the better penetration and entanglement of the polymer chains with that of mucosal layer thereby improving the bioadhesive property. The flexibility of the polymer chains is generally affected by the crosslinking reactions and the hydration of the polymer network. Higher the crosslinking density, lower is the flexibility of the polymer chains.
- Hydration of polymer- In addition to the reduced flexibility of the polymer chains, crosslinking results in the reduced diffusion of water into the crosslinked polymer matrix. Hence highly crosslinked polymeric matrix limits the interpenetration of polymer and mucin chains amongst themselves which in turn results in the decrease in the mucoadhesive strength.
- Hydrogen bonding- In general, stronger the hydrogen bonding stronger is the adhesion. The functional groups responsible for such kind of interaction include hydroxyl, carboxyl and amino groups.
- Charge and degree of ionization of polymer- The presence of charged functional groups in the polymer chain has a marked effect on the strength of the bioadhesion. Anionic polyelectrolytes have been found to form stronger adhesion when compared with neutral polymers.
- Polymer concentration- In general, polymer concentration in the range of 1-2.5 wt % may exhibit sufficient mucoadhesive property for biomedical applications.

### Drug delivery systems

#### 1. Mucoadhesive Drug delivery systems :

Takayama et al have investigated bioadhesive property and the rate of release of a model drug from oral tablet comprising chitosan and sodium hyaluronate(39). It was found that tablets produced from chitosan alone, were less

mucoadhesive than when produced from sodium hyaluronate alone or when using two polymers as a complex. The release rate of drug was highly dependent upon the weight fraction of chitosan in the tablet, with a constant release rate being obtained between 10 and 60 % of chitosan and a rapid increase in release for higher fraction. Thiol-derivative of chitosan improves mucoadhesive properties by the formation of co- valent bonds between thio groups of the polymer and cysteine rich sub domains of glycoproteins in the mucus layer (40). These covalent bonds are supposedly stronger than non-covalent bonds, such as ionic interaction of chitosan with anionic substrates of the mucus layer; this theory was supported by the results of tensile studies with tablets of thiolated chitosan, which demonstrated positive correlation between the degree of modification with thiol bearing moieties and the adhesive properties of the polymer (41).

Marta Roldo et al have evaluated the influence of the degree of modification and the polymer chain length on the mucoadhesive properties and the swelling behavior of the thiolated chitosan derivative obtained via a simple one step reaction between the polymer and 2- iminothiolane. The conjugates differing in molecular mass of the polymer backbone and in amount of immobilized thiol groups were compressed into tablets. They were investigated for their mucoadhesion properties on freshly excised porcine mucosa via tensile studies and rotating cylinder method. The obtained result demonstrated that the total work of adhesion of chitosan-TBA (4-thiobutylamidin) conjugates can be improved by an increasing number of covalently attached thiol groups; 100 fold increase compared to unmodified chitosan was observed for a medium molecular mass chitosan-TBA conjugate exhibiting 264µm thiol groups per gram polymer. Also, the polymer chain length had an influence on the mucoadhesive property of the polymer. The medium molecular mass polymer displayed a four fold improved adhesion on the rotating cylinder compared to the low molecular mass derivative (42).

Miasakinen et al have studied mucoadhesive properties of chitosan

granules to the human esophagus. Chitosan granules were dispensed into gelatin capsules and administered to 10-volunteers. The capsule adhered initially to the distal esophagus (43). The capsule shell started to disintegrate within 5 minutes, about two third of the radioactivity remained detectable in the esophageal region for 1.75 hour. This could be explained on the basis of adherence not only on the gelatin shell but also chitosan granules to the esophageal mucosa. Mia Sakkinen et al have studied in vivo evidence of weather microcrystalline chitosan formulation acted as gastro retentive system in humans by gamma scintigraphic evaluation of the fate of microcrystalline chitosan granules in human stomach (44). It was concluded that the in vivo mucoadhesion of the formulation was erratic, and the formulation studied were not reliable for gastro retentive drug delivery systems

### **2. Ophthalmic drug delivery system:**

Chitosan because of its low toxicity exhibits favorable biological behavior, such as bioadhesion, permeability enhancing properties and interesting physico-chemical characteristics, which make it a unique material for the design of ocular drug delivery vehicles (45). The potential of chitosan-based systems is for improving the retention and biodistribution of drugs applied topically onto the eye. Chitosan based formulations for ophthalmic drug delivery are chitosan gels, chitosan-coated colloidal systems, and chitosan nanoparticles.

Chitosan-based colloidal systems are found to work as transmucosal drug carriers, either facilitating the transport of drugs to the inner eye (chitosan-coated colloidal systems containing indomethacin) or their accumulation into the corneal/ conjunctival epithelia (chitosan nanoparticles containing cyclosporin) [46]. The micro-particulate drug-carrier (micro-spheres) is a promising means of topical administration of acyclovir to the eye

### **3. Nasal drug delivery system:**

The nasal mucosa presents an ideal site for bioadhesive drug delivery systems. Chitosan based drug delivery systems, such as micro spheres, liposomes and gels have

been demonstrated to have good bioadhesive characteristics and swell easily when in contact with the nasal mucosa increasing the bioavailability and residence time of the drugs to the nasal route. Various chitosan salts such as chitosan lactate, chitosan aspartate, chitosan glutamate, and chitosan hydrochloride are good candidates for nasal sustained release of vancomycin hydrochloride (47).

Nasal administration of Diphtheria Toxoid (DT) incorporated into chitosan microparticles results in a protective systemic and local immune response against DT with enhanced IgG production. Nasal formulations have induced significant serum IgG responses similar to secretory IgA levels, which are superior to parenteral administration of the vaccine (48). Research showed bioadhesive chitosan microspheres of pentazocine for intranasal systemic delivery significantly improved the bioavailability with sustained and controlled blood level profiles compared to intravenous and oral administration (49). Nasal absorption of insulin after administration into chitosan powder were found to be the most effective formulation for nasal drug delivery of insulin in sheep compared to chitosan nanoparticles and chitosan solution.

### **4. Buccal drug delivery system:**

An ideal buccal delivery system should stay in the oral cavity for few hours and release the drug in a unidirectional way toward the mucosa in a controlled or sustained-release fashion. Mucoadhesive polymers prolong the residence time of the device in the oral cavity (50), while bilayered devices ensure the release of the drug occurs in a unidirectional way. Chitosan is an excellent polymer to be used for buccal delivery because it has muco/bioadhesive properties and can act as an absorption enhancer (51). Directly compressible bioadhesive tablets of ketoprofen containing chitosan and sodium alginate in the weight ratio of 1:4 showed sustained release 3 hours after intraoral (into sublingual site of rabbits) drug administration(52). Buccal tablets based on chitosan microspheres containing chlorhexidine diacetate gives prolonged release of the drug in the buccal cavity improving the antimicrobial activity of the

drug (53). Chitosan microparticles with no drug incorporated have antimicrobial activity due to the chitosan. The buccal bilayered devices (bilaminated films, palavered tablets) using a mixture of drugs (nifedipine and propranolol hydrochloride) and chitosan, with or without anionic crosslinking polymers (polycarbophil, sodium alginate, gellan gum) has promising potential for use in controlled delivery in the oral cavity (54). Bioadhesive tablets of nicotine containing 0% to 50% w/w glycol Chitosan gives good adhesion

#### 5. Vaginal drug delivery system:

Chitosan, modified by the introduction of thioglycolic acid to the primary amino groups of the polymer, embeds clotrimazole, an imidazole derivative, is widely used for the treatment of mycotic infections of the genitourinary tract. By introducing thiol groups, the mucoadhesive properties of the polymer are strongly improved and this is found to increase the residence time of the vaginal mucosa tissue (26 times longer than the corresponding unmodified polymer), guaranteeing a controller drug release in the treatment of mycotic infections(55). Vaginal tablets of chitosan containing metronidazole and acriflavine have showed adequate release and good adhesion properties(56)

#### CONCLUSION

Current use of mucoadhesive polymers to increase contact time for a wide variety of drugs and routes of administration has shown dramatic improvement in both specific therapies and more general patient compliance. Hence mucoadhesive polymers can be used as means of improving drug delivery through different routes like gastrointestinal, nasal, ocular, buccal, vaginal and rectal. Many potential mucoadhesive systems are being investigated which may find their way into the market in near future.

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