CHITOSAN: A MUCOADHESIVE POLYMER

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ABSTRACT
Chitosan is polymer having mucoadhesion properties. Being biological origin it have less toxic effect than other polymers. Present article review on various physicochemical biological properties, and its method of preparation. Chitosan have the property to get modified or make the complex with other excipients leads to enhancing its properties. Chitosan is a versatile natural polymer. This article reviewed different aspects of Chitosan with their applicability in pharmaceutical formulations. This review emphasized that research on Chitosan based systems containing various drugs for various therapeutic applications have increased in recent years. So this article has fulfill the requirement of a review on this naturally derived polymer in present scenario.

KEYWORDS: Chitosan, mucoadhesion, formulation.

INTRODUCTION
Polymers
Polymers are macromolecules composed of repeating structural units of monomers concluded by covalent chemical bonds and their process is known as polymerization. Polymers can be classified as natural or synthetic polymers. Natural polymer for example protein (Collagens, silk, and keratin), carbohydrate (Starch, glycogen) are widely used materials for conventional and novel dosage form. These materials are inert, nontoxic, less expensive, biodegradable, eco friendly and widely available.
Chitosan
Chitosan is no longer considered as just a waste product from seafood industries. This material is now being utilized by industry to solve problems and to improve existing products as well as create new ones.\(^1\)

Officially, chitosan having non-proprietary names are according to BP, chitosan hydrochloride, and according to the Ph Eur, chitosan hydrochloridum. Chitosan having synonyms as 2-Amino-2-deoxy-(1,4)-β-D-glucopyranan; deacetylated chitin; deacetylchitin; β -1,4-poly-D-glucosamine; poly- D-glucosamine; poly-(1,4- β - D-glucopyranosamine).

Chemical Name and CAS Registry Number
Poly-β -(1,4)-2-Amino-2-deoxy-D-glucose [9012-76-4].

Empirical Formula and Molecular Weight
Partial deacetylation of chitin results in the production of chitosan, which is a polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine. Chitosan is the term applied to deacetylated chitins in various stages of deacetylation and depolymerization and it is therefore not easily defined in terms of its exact chemical composition. A clear nomenclature with respect to the different degrees of N-deacetylation between chitin and chitosan has not been defined, and as such chitosan is not one chemical entity but varies in composition depending on the manufacturer. In essence, chitosan is chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be greater than 80–85%. Chitosan is commercially available in several types and grades that vary in molecular weight by 10000–1000000, and vary in degree of deacetylation and viscosity.

Chitosan occurs as odourless, white or creamy-white powder or flakes. Fibre formation is quite common during precipitation and the chitosan may look 'cottonlike'.\(^2\) The pharmaceutical specification for chitosan is given in Table 1.

Table 1: Pharmacopeial specifications for chitosan.\(^2\)

<table>
<thead>
<tr>
<th>Test</th>
<th>PhEur 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification</td>
<td>+</td>
</tr>
<tr>
<td>Characters</td>
<td>+</td>
</tr>
<tr>
<td>Appearance of solution</td>
<td>+</td>
</tr>
<tr>
<td>Matter insoluble in water</td>
<td>≤ 0.5%</td>
</tr>
<tr>
<td>pH (1% w/v solution)</td>
<td>4.0–6.0</td>
</tr>
</tbody>
</table>
Chitin is the second most abundant natural polymer in nature after cellulose and it is found in the structure of a wide number of invertebrates (Crustaceans, exoskeleton, and insect cuticles) and cell wall of fungi.

Naturally chitosan occurs only on some fungi (Mucoraceae). Commercially chitosan prepared by the chemical deacetylation of chitin from crustacean source under alkaline condition. Chitin is a beta (1-4) - 2- acetamido-2-deoxy-D-glucopyranose as a repeating unit.\[3\]

**Structure**

Structure of chitin and chitosan is shown in Figure 1

![Fig.1, Structure of chitin and chitosan](image)

The percentage of chitin content varies with the source of supply. Industrially, the isolation of chitin from crustacean shell mainly involves removal of proteins and dissolution of calcium carbonate which present in crab shell in high concentration.

Chitin is water insoluble and insoluble in almost all organic solvents.[4]

**Preparation methods of chitosan from chitin**

1. **Chemical manufacturing of chitin and chitosan**

Chitosan is manufactured commercially by chemically treating the shells of crustaceans such as shrimps and crabs. The basic manufacturing process involves the removal of proteins by treatment with alkali and of minerals such as calcium carbonate and calcium phosphate by treatment with acid. Before these treatments, the shells are ground to make them more

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity</td>
<td>+</td>
</tr>
<tr>
<td>Degree of deacetylation</td>
<td>+</td>
</tr>
<tr>
<td>Chlorides</td>
<td>10.0–20.0%</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>≤ 40 ppm</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>≤ 10%</td>
</tr>
<tr>
<td>Sulfated ash</td>
<td>≤ 1.0%</td>
</tr>
</tbody>
</table>
accessible. The shells are initially deproteinized by treatment with aqueous sodium hydroxide 3–5% solution. The resulting product is neutralized and calcium is removed by treatment with an aqueous hydrochloric acid 3–5% solution at room temperature to precipitate chitin. The chitin is dried so that it can be stored as a stable intermediate for deacetylation to chitosan at a later stage. N-deacetylation of chitin is achieved by treatment with an aqueous sodium hydroxide 40–45% solution at elevated temperature (110°C), and the precipitate is washed with water. The crude sample is dissolved in acetic acid 2% and the insoluble material is removed. The resulting clear supernatant solution is neutralized with aqueous sodium hydroxide solution to give a purified white precipitate of chitosan. The product can then be further purified and ground to a fine uniform powder or granules. The animals from which chitosan is derived must fulfil the requirements for the health of animals suitable for human consumption to the satisfaction of the competent authority. The method of production must consider inactivation or removal of any contamination by viruses or other infectious agents.[2]

2. Biotechnological method for chitin and chitosan

Shellfish waste obtained from crab shrimp etc were grind in the presence of water. Resulting substance demineralised with the help of *Pseudomonas argenosa F722*. After demineralization; obtained product get deproteinized by using enzyme or proteolytic bacteria. After enzymatic degradation, enzyme should be deactivated. Deproteinized product washed it and then dried to obtained chitin. Chitin is then deacetylated by using chitin deacetylase or lactic acid bacteria or by using hydrolysis using chitinolytic enzyme which produces chitosan on washing and drying.

3. Biological process for chitin and chitosan production

Crustacean shell obtained from crab, shrimp etc. It is demineralised with the help of organic acid producing bacteria. Then it is deproteinized by protease producing bacteria to produce chitin. Chitin is then deacetylated in the presence of chitin deacetylase to produce Chitosan.[5]

Properties of chitosan

1. Physicochemical Properties

Chitosan is solid state, semicrystalline polymer. Chitosan is broadly classified as chitosan oligomer having 2 monomer unit and chitosan polymer with more than 12 monomers which further classified as chitosan polymer having low molecular weight greater than 150 KDa, medium molecular weight in between 150-700 KDa, and High molecular weight 700 – 1000
KDa. It is notoxic, biodegradable, biocompatible, citocompatabilial, mucoadhesive, haemostatic, analgesic, adsorption enhancer, antimicrobial activity, anticholeroleolemic activity, angiogenesis, granulation and scar formation, macrophage activation. The degree of deacetylation of typical commercial chitosan is usually ranged between 66 – 95%. In solid state, chitosan is a semicrystalline in nature which exhibit polymorphism. As the removal of acetyl moieties that are present in the amine group.

**SOLUBILITY**

Chitosan is soluble in acidic medium and poor soluble in neutral and basic pH. Soluble in dilute organic acid solution such as acidic acid, formic acid, succinic acid and lactic acid at pH below 6.5. Low solubility at pH 7.4 and higher pH. Solubilisation occurs through protonation of -NH$_2$ group of D-glucosamine derivative which give polycation in acidic medium.

a. Chitosan with more than 50% deacetylation soluble in acidic medium

b. Chitosan with 50% deacetylation soluble in neutral medium

c. Chitosan with less than 50% deacetylation soluble in aqueous medium at pH 9.

The solution properties of chitosan are depends upon distribution pattern of acetyl group along the main chain and mol. wt. The concentration of the CH+ needed to dissolve chitosan must at least be equal to conc. of NH$_2$ unit participating in solubilisation process. In the presence of excessive HCl salting out of the chitosan takes place. The aqueous solubility of chitosan is greatly influence by the addition of electrolyte to the solution. It forms the extended conformation in the solution form of chitosan. It is due to repulsion of positively charge deacetylated unit on the neighbouring glucosamine units. Addition of electrolyte reduces the interchain repulsion and induces a more random coil like conformation in the molecule which eventually results in salting out and precipitation of chitosan.

**pKa** - 6.2 - 7

**Acidity/alkalinity:** pH = 4.0–6.0 (1% w/v aqueous solution)

**Density:** 1.35–1.40 g/cm$^3$

**Glass transition temperature:** 203°C

**Moisture content:** chitosan adsorbs moisture from the atmosphere, the amount of water adsorbed depending upon the initial moisture cont ent and the temperature and relative humidity of the surrounding air.

**Particle size distribution:** <30 μm,2
VISCOSITY

The viscosity of the chitosan greatly affected by molecular weight, ionic strength, pH, and temperature of the solution. The viscosity of the solution increases with increase in concentration of the chitosan and degree of deacetylation, but decrease in temperature and pH. It possesses pseudoplastic behaviour with solution viscosity decreases with an increase in shear rate. Typical viscosity values are shown in Table 2.

Table 2: Typical viscosity (dynamic) values for chitosan 1% W/V solutions in different acids.

<table>
<thead>
<tr>
<th>Acid</th>
<th>1% acid concentration</th>
<th>5% acid concentration</th>
<th>10% acid concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Viscosity (mPa)</td>
<td>pH</td>
<td>Viscosity (mPa)</td>
</tr>
<tr>
<td>Acetic</td>
<td>260</td>
<td>4.2</td>
<td>260</td>
</tr>
<tr>
<td>Adipic</td>
<td>190</td>
<td>4.11</td>
<td>-</td>
</tr>
<tr>
<td>Citric</td>
<td>35</td>
<td>3.0</td>
<td>195</td>
</tr>
<tr>
<td>Formic</td>
<td>240</td>
<td>2.6</td>
<td>185</td>
</tr>
<tr>
<td>Lactic</td>
<td>235</td>
<td>3.3</td>
<td>235</td>
</tr>
<tr>
<td>Malic</td>
<td>180</td>
<td>3.3</td>
<td>205</td>
</tr>
<tr>
<td>Malonic</td>
<td>195</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>Oxalic</td>
<td>12</td>
<td>1.8</td>
<td>100</td>
</tr>
<tr>
<td>Tartaric</td>
<td>52</td>
<td>2.8</td>
<td>135</td>
</tr>
</tbody>
</table>

Chitosan is known to possessed good complexing capacity. It has good complexing capacity. The presence of C-NH₂ group involved in specific interaction with metal. A higher degree of chitosan is attained with chitosan characterized by greater degree of deacetylation. The affinity of chitosan for divalent and trivalent cation of chloride salts as

Cu²⁺ >> Hg²⁺ >> Zn²⁺ >> Cd²⁺ > Ni²⁺ > Co²⁺ - Ca²⁺ > Eu³⁺ > Nd³⁺ > Cr³⁺ - Pr³⁺.

Chitosan is able to form electrostatic complexes under acidic condition with oppositely charged surfactant (SLS). Chitosan can complex with oppositely charged polymer such as polyacrylic acid, Na salt of polyacrylic acid, CMC, xanthum, carrageenan, alginate, pectin, heparin, hyluronan, sulfonated cellulose, dextron, and chondrotoin sulfate. Chitosan may be cross linked with reagent such as epichlorhydrin, genipin, disocynate and 1, 4- butandiol diglycidyl ether. Starch chitosan blend can be cross linked through oxidizing the starch to produce polyaldehyde which react with NH₂ in the presence of reducing agent.

Chitosan form the hydrogel when polymer react with multivalent amino such as glycerol phosphate, oxalic acid, pyrophosphate, tripolyphosphate, tetraposphate, hexaphosphate, metaphosphate and Fe (CN6)⁴⁻ / Fe (CN6)⁻³.
1. Biological Properties
It is a natural, biodegradable, biocompatible, degraded by lysosome or enzyme chitinase producing N- acetyl glucosamine. Chitosan have low level of toxicity. LD50 values found to be in excess of 16gm/kg body wt. of mouse, higher than that of sucrose. Low deacetylated have high toxicity than high deacetylated. Chitosan is lack of irritant and allergic effect. It has immunostimulant properties. It have high capacity to adhere to mucosa, owing to ionic interaction between NH$_2$ and negatively charged mucus gel layer, it enhances transmucosal drug absorption and is known to reversible membrane damage. The primary mechanism of adhesion at the molecule level is affected via electrostatic attraction. Higher mol.wt. of chitosan 1400kDa produces higher mucoadhesion than low mol. wt.

Modification of chitosan
1. Quaternization of NH$_2$ Group
Solubility of chitosan can be increased by quarterisation. Chitosan have NH$_2$ group, on quarterisation it form no. of derivative such as

1. O, N carboxy methyl chitosan – The NH- group of chitosan is reacted with carbonyl group of aldehyde and glyoxylic acid and then hydrogenated by the reaction with NaBH4 or NaCNBH3 to give N carboxy methyl chitosan. Another method is solubilised chitosan with 1% acetic acid solution which get approximately 1-1.5 %w/v solution. This solution is treated with glyoxylic acid in molar ration 1:1 to 1:3 followed by reduction with portions of sodium borohydride to obtain pH of 4-5 without precipitation in reaction mixture. The viscous solution is then dialyzed against water and lyophilized to get N-carboxymethyl chitosan. Use of the reductive alkylation process gave a product having approximately 70% N,N-dicarboxymethyl chitosan units.$^6$ The chitosan is soaked in alkaline solution at freezing or room temperature for 2-24 h. The concentration of chitosan for this purpose commonly reported is 4% -20% w/v in 40-50% w/v solution of NaOH. The activated chitosan is then reacted with monochloroacetic acid in solid or solution form. The concentration of monochloroacetic acid used is 1:1 -1:6 by weight in isopropanol/ethanol and reaction is carried at 0–60°C for 2–24 h. The product is precipitated by solvent as acetone, ethanol and desalted by pH adjustment or dialysis.$^7$$^8$$^9$

2. Chitosan 6-o-sulfate: Chitosan (65000 D) (1.0 g) was suspended in 50 ml methanol with stirring at room temperature, then octaldehyde (1.02 g) was added. After 24 h, KBH4 (0.5 g) dissolved in 5 ml water was slowly added to the solution. After a further 24 h continuous
stirring, the reaction solution was neutralized with 2N hydrochloric acid and the product was precipitated with methanol. The precipitate was filtered and repeatedly washed with methanol and water. The product, N-octylchitosan (OC1) was dried under vacuum at 60 - 80°C overnight. OC2 was prepared with chitosan (25000 D) as the starting material using the same method as above. Different N-alkyl substituents DC1 (chitosan 65000 D), DC2 (chitosan 25000 D), LC1 (chitosan 65000 D) and LC2 (chitosan 25000 D) were synthesized using decanal and lauril aldehyde, respectively. OC1 (1.05 g) was suspended in DMF (40 ml), and magnetically stirred overnight. Chlorsulphonic acid (20 ml) was added dropwise into DMF (40 ml) with stirring at 108°C under N2 atmosphere. After completely dripped, the solution was kept in agitation for 1 h, and then the suspension of OC1 and DMF was added to the above solution. The mixture was reacted at 108°C under N2 atmosphere for 24 h. The reaction solution was neutralized with 20% NaOH to pH 7, and the filtered solution was dialyzed (MWCO 10000) against distilled water, then lyophilized and the OCS1 powder was obtained. Different chitosan derivatives, OCS2, DCS1, DCS2; LCS1, LCS2 were obtained using the same method.

3. Trimethyl chitosan ammonium: N,N,N-trimethyl chitosan (TMC) is a cationic polyelectrolyte obtained by extensive methylation of chitosan parent polymer.\textsuperscript{[11]} The resulting derivative is a water-soluble polysaccharide useful for a variety of applications. The TMC was obtained by methylation of chitosan with dimethylsulfate at 70°C. Reaction is carried out as 1.0 g of chitosan (0.005 mol) in 16 mL of dimethylsulfate and 4 mL of deionized water.\textsuperscript{[12],[13]} Methylation leads to increase solubility of chitosan in water at neutral and basic pH values. The increase in solubility is achieved by replacing the primary amino group on the C-2 position of chitosan with quaternary amino groups\textsuperscript{[14],[15]} TMC was investigated for permeation enhancing properties and toxicity, using the Caco-2 cells as a model for intestinal epithelium. Synthesis of TCM is shown in Figure2.

![Fig. 2, Synthesis of N-Trimethyl chitosan chloride (TMC).\textsuperscript{[16]}](image)

5. Carbohydrate branched chitosan- By reductive alkylation carbohydrates can be grafted on the chitosan backbone at the C-2 position: Disaccharides (celllobiose, lactose, etc.) having
a reducing end group, are introduced, in the presence of a reluctant, on chitosan in the open chain form.\textsuperscript{[17][18]} These derivatives are water soluble. Carbohydrates can also be introduced without ring opening on the C-6 position.\textsuperscript{[19][20]} These derivatives are important as they are recognized by the corresponding specific lectins and thus could be used for drug targeting.\textsuperscript{[21],[22]}

6. Polyethylene grafted chitosan - Poly(ethylene glycol) (PEG) is a highly water-soluble, amphipathic polymer and frequently used for chemical modification of natural and artificial macromolecules for biomedical applications. Grafting PEG onto chitosan should be a promising approach to obtain water-soluble chitosan derivatives. 6-O-triphenylmethylchitosan was prepared from chitosan by the method reported by Kurita et al. PEG-g-chitosan was prepared according to the following methods.

The coupling reaction of 6-O-triphenylmethyl-chitosan with MeO-PEG acid was carried out by using the water-soluble carbodiimide (WSC)-hydroxybenzotriazole (HOBO method in N,N-dimethylformamide (DMF) to give PEG-grafted-6-O-triphenylmethyl-chitosan. The reaction mixture was subjected to gel-filtration chromatography (column: Sephadex LH-60, 2 × 100 cm, eluent: DMF) monitored by UV detector at 265 nm to afford the conjugate. The high molecular weight fraction was separated and evaporated under reduced pressure. The yellow solid obtained was treated by 50% (v/v) acetic acid for 2h to deprotect its triphenylmethyl groups. Then the acidic solution was neutralized with triethylamine (TEA), and dialyzed in cellulose tube against water, and freeze-dried to give the objective PEG-g-chitosan. Deprotection of triphenylmethyl groups was confirmed by disappearance of absorption at 265 nm using UV spectrophotometer. The purity of PEG-g-chitosan obtained was confirmed by gel-permeation chromatography (GPC) (column: Shodex OH pack SB-803, Showa Denko, eluent 1/15 M phosphate buffer, standard: pullulan) monitored by refractive index (r.i.) detector.\textsuperscript{[23]}

7. Cyclodextrin linked chitosan - To a solution of chitosan (200 mg) in 0.2 M acetate buffer at pH 4.4 (100 ml) was added a solution (50 ml) of 2 that was prepared form 1 (1 g) was added by portions. The mixture was stirred for 1h at room temperature. Sodium cyanoborohydride (260 mg) was added to the resulting solution. The mixture was stirred for 4 days at room temperature, neutralized with 5% ammonia water, subjected to ultrafiltration through a membrane, and lyophilized to give the 3-CD-linked chitosan.\textsuperscript{[24]}
Strategies in chitosan derivative

1. Functional group modification

a. Thiolated chitosan - Chitosan when treated with thioglycolic acid, it form thiolated chitosan shown in Figure 3.

![Fig. 3, Thiolated polymers.][26]

It has better mucoadhesion and permeation enhancing properties in oral drug delivery. Thiolated polymer can form disulfide bonds between the thiomer and the mucus gel layer takes place either via thiol/disulfide exchange reactions or via a simple oxidation process of free thiol groups. The different types of mucus glycolproteins or designated mucins exhibiting cysteine-rich subdomains have been reviewed previously. Mechanism of disulfide bond formation between thiomers and mucus glycoproteins (mucins) is shown in Figure 4.

![Fig. 4, Mechanism of disulfide bond formation between thiomers and mucus glycoproteins (Mucins).[26]]

b. N- acetylated chitosan - Generally useful in gene drug delivery. Fully de-N-acetylated chitosan oligomers (dimer to hexamer) were prepared by nitrous acid, depolymerization of chitosan takes place and separated by size exclusion chromatography (SEC). The oligomers were then stored in aqueous 0.15M ammonium acetate solution at pH 4.5 until the time of the
polymerization reaction. The de-N-acetylated dimer to hexamer fractions were each polymerized (self-branched) by reductive N-alkylation of a 1% solution at pH 5.5 in 0.15M ammonium acetate and 0.1M NaCl. NaCNBH$_3$ was added in excess (approx. 50 mg) twice, after 30 min and 24 h. The reaction was stopped after 4 days on stirring in room temperature by adding concentrated HCl (pH < 2) to remove NaCNBH$_3$. The products were then lyophilized after adjusting the pH< 4 to obtain the derivatives in their HCl form. Following scheme is given for nitrous acid degradation and subsequent reductive N-alkylation produce branched chitosan. The process is shown in Figure 5.

Fig. 5, Nitrous acid degradation and subsequent reductive N-alkylation produce branched chitosan.$^{[27]}$

2. Copolymerization - A 250 ml three necked round bottomed flask, filled with a magnetic stirrer, thermometer, and reflux condenser in a temperature-controlled oil bath, was used for the graft reaction. Firstly, a desired quantity of chitosan was dissolved in 1 wt% of acetic acid aqueous solution at 60°C. The total volume of the aqueous solution was 80 ml in all experiments. After the chitosan was fully dissolved, temperature of the system was strictly controlled at a required value. Then ammonium persulfate powder was added into the solution. The mixture was continuously stirred at the desired temperature until completion of the copolymerization reaction. At the end of the graft copolymerization, the mixture was continuously stirred for 15 min at room temperature, and then neutralized to pH 10 to precipitate the product with 1 M NaOH solution. Then the mixture was centrifuged to obtain the solid. The solid mixture was washed by the distilled water and centrifuged repeatedly to pH 7, then washed by anhydrous alcohol to remove the salts and centrifuged for 2 times. The
centrifugate was dried at 70°C to a constant weight. After filtration and washed by DMF for several times, the pure chitosan-g-polyacrylonitrile (CS-g-PAN) was obtained by thoroughly washed with anhydrous alcohol, and dried at 70°C to reach a constant weight. All the samples were absolutely dried before used for characterization. Grafting rate (%G), which designates the amount of polymer grafted on the substrate backbone (chitosan), and efficiency of grafting (%E), which indicates the efficiency of conversion of the initial polymer to the grafted PAN, were calculated from the increase in weight of the chitosan after graft copolymerization \([28]\):

\[
\% \text{Grafting (%G)} = \frac{W_1 - W_0}{W_0} \times 100
\]

\[
\% \text{Efficiency (%E)} = \frac{W_1 - W_0}{W_2} \times 100
\]

Where W1, W0 and W2 denote the weight of the grafted chitosan, the weight of original chitosan and weight of the monomer used, respectively. The experiments of graft polymerization were performed with two parallel experiments, and the data was the average value of the two results. When the two data were quite different, we carried out the third one to confirm it.

**Mucoadhesion properties**

Chitosan structure possesses cationic group, due to this group it has mucoadhesive property. Chitosan mainly combine with anionic group of mucus i.e sialic acid and sulfonic group substituent. Hence ionic interaction is take place in between cationic primary amino acid group of chitosan with anionic sialic acid group of mucus, mucoadhesion can be achieved. In addition, hydrophobic interactions might contribute to its mucoadhesive properties. In comparison with various anionic polymeric excipients such as carbomer, polycarbophil, and hyaluronic acid, however, its mucoadhesive properties are weak.\([29]\) To achieve better mucoadhesive properties, polymer should have cohesive properties as that of adhesive. If not then polymer fails to achieve mucoadhesion. Chitosan has weak cohesive properties but can be improved by formation of complexes with multivalent anionic drug or other anionic excipients.\([30]\) Trimethylation of the primary amino group of chitosan provides an even more cationic character of the polymer. When trimethylated chitosan (TMC) is additionally PEGylated, its mucoadhesive properties are even up to 3.4-fold improved.\([31]\) Due to the immobilization of thiol groups on chitosan, its mucoadhesive properties can also be strongly improved, as the thiolated polymer is capable of forming disulfide bonds with mucus glycoprotein of the mucus gel layer, placing it among the most mucoadhesive polymers.
known so far. \[32\] In addition, as inter- and intrachain disulfide bonds are also formed within chitosan itself, thiolated chitosan exhibits substantially improved cohesive properties. Recently, the mucoadhesive properties of thiolated chitosan were even significantly further improved by the preactivation of thiol groups on chitosan via the formation of disulfide bonds with mercaptonicotinamide.\[33\]

Application in the drug delivery

1. In conventional dosage form - It is used as binder, disintegrating agent, and coating material. It has been formulated as core with acrylic coat in design of enteric coated.
2. Chitosan form a gel at low pH and exhibit antacid or antiulcer properties which prevent or alleviate gastric discomfort induced by drug irritation.\[34\]
3. Chitosan dosage forms are given in Table 3.

Table 3 : List of chitosan based formulations prepared by different methods

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Dosage form</th>
<th>Method of preparation</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tablets</td>
<td>Matrix Coating</td>
<td>Theophylline,[35] Propranolol HCl, [36]</td>
</tr>
<tr>
<td>2</td>
<td>Capsules</td>
<td>Hard gelatine shell</td>
<td>Insulin [37]</td>
</tr>
<tr>
<td>3</td>
<td>Microspheres/</td>
<td>Emulsion cross-linking, Coacervation/</td>
<td>Gentamicin Sulphate,[38] Propranolol-HCl,</td>
</tr>
<tr>
<td>3.</td>
<td>Microparticles</td>
<td>precipitation Spray-drying</td>
<td>Cimetidine,[40]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ionic gelation</td>
<td>Bovine serum albumin (BSA), [41]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sieving method</td>
<td>Bovine serum albumin, Clozapine, [42]</td>
</tr>
<tr>
<td>4</td>
<td>Nanoparticles</td>
<td>Emulsion-droplet coalescence, Coacervation/ precipitation Ionic gelation Reverse micellar method</td>
<td>Gadopentetic acid, [43] Bovine serum albumin, [44] Ascorbic acid, [45] Doxorubicin [46]</td>
</tr>
<tr>
<td>5</td>
<td>Beads</td>
<td>Coacervation/ precipitation</td>
<td>Bovine serum albumin, Insulin [47]</td>
</tr>
<tr>
<td>6</td>
<td>Films</td>
<td>Solution casting</td>
<td>Ofloxacin [48]</td>
</tr>
<tr>
<td>7</td>
<td>Gel</td>
<td>Cross-linking</td>
<td>5-Fluorouracil [49]</td>
</tr>
</tbody>
</table>

Various chitosan based drug delivery systems

1. Oral drug delivery

As liquid formulations are not favoured by patients and storage stability problems of drugs such as peptide drugs have to be overcome, solid dosage forms are the delivery systems of choice. Apart from capsules, tablets are therefore the likely most favourable dosage form. As tablets provide an accurate dosage and are easy to manufacture and handle, numerous drug delivery systems comprising chitosan are based on this type of dosage form. The drug can thereby be homogenized with chitosan and directly compressed to tablets. As chitosan
precipitates at pH above 6.5, it loses its mucoadhesive and permeation enhancing properties in distal segments of the intestine. This effect reduces its applicability to drugs having their absorption window in the proximal segment of the GI tract. \cite{33} Dhaliwal et al. for instance, could improve the oral bioavailability of acyclovir 3-fold and 4-fold due to the incorporation of this drug in chitosan and thiolated chitosan, respectively.\cite{50}

2. Ocular drug delivery
A model drug Cyclosporin A (CyA) was chosen. Chitosan and CyA is complexed in the form of nanoparticle. Under sink condition in vitro studies are carried out which revealed that an initial burst release of the drug followed by gradual drug release up to 24 h. For in vivo studies rabbits are chosen and CyA loaded chitosan nanoparticles instilled topically (i.e., cornea and conjunctiva). These levels were significantly higher than those obtained following the instillation of CS solution containing CyA that CS nanoparticles could be used as a vehicle to and aqueous CyA suspension. The study indicated enhances the therapeutic index of the clinically challenging drugs with potential application at the extra ocular level.\cite{51}

3. Nasal Drug Delivery
Diabetes is routinely treated by injection of insulin. Insulin can administered via other route of administration such as oral as well as nasal. But insulin has enzymatic degradation problem and poor transmucosal absorption. So there is to need to develop new formulation which includes insulin and excipients into dosage form, increase in physical stability and bioavailability of insulin. Chitosan and its derivatives or salts have been widely investigated as functional excipients of delivering insulin via oral, nasal and transdermal routes. Characteristic of chitosan for its mucoadhesive and able to protect the insulin from enzymatic degradation, prolong the retention time of insulin, as well as, open the inter-epithelial tight junction to facilitate systemic insulin transport. The chitosan can be employed to strengthen the physicochemical stability of insulin and multi-particulate matrix. On modification of chitosan chemically, produces water soluble low molecular weight polymer protect the insulin molecule and sulphated chitosan which markedly opens the paracellular channels for insulin transport. Nanoparticles of chitosan and fatty acid as hydrophobic molecule promote the insulin absorption via lymphoid tissue. Attainment of optimized formulations with higher levels of pharmacological bioavailability is deemed possible in future through targeted delivery of insulin using chitosan with specific adhesiveness to the intended absorption mucosa.\cite{4}
4. Vaginal drug delivery
In the treatment of mycotic infection of genitourinary tract clotramazole is embedded in modified chitosan thioglycolic acid derivatives. By introducing thiol groups, the mucoadhesive properties of the polymer were strongly improved, and this resulted in an increased residence time of the vaginal mucosa tissue (26 times longer than the corresponding unmodified polymer). Vaginal tablets of chitosan containing metronidazole, acriflavine, and other excipients showed adequate release and good adhesion properties.\[52]\[53]

5. Buccal drug delivery
Biological properties of chitosan allow drug absorb through oral mucosa. Oral mucosal bioadhesive tablets of diltiazem were prepared by directly compressing the drug with a mixture of chitosan and sodium alginate. In vitro adhesion studies indicated adhesion properties comparable to those of a commercial formulation.\[54]\[55]

6. Parenteral drug delivery
Chitosan can administered intravenously, which has low molecular weight and high purity, its use in injectable preparations has received considerable attention within the last years. In controlled release technology, biodegradable polymeric carriers offer potential advantages for prolonged release of low-molecular-weight compounds to macromolecular drugs.\[55]\[56]

7. Targeted drug delivery
Chitosan has unique physicochemical and biological properties so it is safe and effective in drug delivery system. Chemical modification of chitosan is due to presence of primary hydroxyl and amine groups located on the backbone of chitosan to control its physical properties. Chitosan conjugated with hydrophobic molecule, forming amphiphilic molecule which may self assembled nanoparticle having encapsulating efficiency which deliver drug to site specific. By chemical conjugation with drug form prodrug, exhibiting the appropriate biological activity at the target site. Mucoadhesive and absorption enhancement properties of chitosan increase the in vivo residence time of the dosage form in the gastrointestinal tract and improve the bioavailability of various drugs.\[56]\[57]

8. Gene therapy
Chitosan-based gene delivery systems have been proven to be effective for non-viral gene therapy.\[57]\[58] The chitosan–DNA complexes are very easy to synthesise and are more effective
compared to the commonly used polygalactosamine – DNA complexes, but, their use is limited because of the lower transfection efficiency.\cite{58}

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