Chitosan and its Role in Ocular Therapeutics

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Abstract: From the past few decades, tremendous awareness has been laid on the use of natural polymers in ocular drug delivery. Chitosan, a modified natural carbohydrate polymer, has number of applications in the field of ophthalmics and attracted a great deal of attention of scientific community, academicians and environmentalists due to its unique features. Chitosan has been explored for the delivery of drugs, genes, biotechnological products, proteins and peptides to the target site within ocular tissues. Chitosan being a polycationic in nature interacts with the polyanionic surface of ocular mucosa through hydrogen bonding /ionic interactions and enhance the mucoadhesive effect of formulation. Sustained and controlled ocular delivery can be achieved with chitosan based formulations like chitosan gels, inserts, chitosan coated liposome/niosome and chitosan nanoparticles. This review discussed various aspects related to chitosan and chitosan based formulations particularly developed for ocular therapeutics. The fate and toxicological consideration related to chitosan, resulting with its interaction to ocular tissues, has also been summed up in addition.

Keywords: Chitosan; ocular delivery; nanoparticles; ocular toxicity.

1. INTRODUCTION

Recent efforts have been directed towards the development and fabrication of dosage form based on safe and effective biodegradable polymer, particularly of natural origin in pharmacology. Chitosan, due to its unique advantages and biological properties has become one of the best candidates to be used in biomedical and pharmaceutical research areas [1]. Chitosan is the second most abundant polysaccharide found in nature next to cellulose. The physico-chemical properties of chitosan show pronounced effect on its stability and residence time [2]. They make it highly valuable and useful in ocular drug delivery. Polycationic character and bioadhesive nature of chitosan enhances the drug residence time in ocular tissues by interaction with the anionic surfaces of mucin residues [3]. Chitosan has been used as a carrier for the delivery of different class of ocular therapeutic molecules such as antibiotics, anti-inflammatory, anticholinergics, analgesics and genes etc. Ocular delivery is one of the most delicate areas of research as eye is a small sense organ having a multi-compartmental system [4]. Traditional/conventional systems that are used in ocular delivery are ineffective in prolonging residence period of bioactive and suffer with the problems such as washing out, undesired systemic absorption and low drug contact time with the corneal surface. Chitosan shows good muco-adhesive and biodegradable property as screened by scientists and hence can be used for controlled drug delivery particularly for opthalmological applications [5] as it prolongs the residence time of drug in the tear film and improved the patient compliance [6] too.

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2. CHEMISTRY AND SOURCES OF CHITOSAN

Chitosan (2-amino-2-deoxy-D-glucose) is a polycationic polymer which is obtained from chitin (β linked N-acetyl- D-glucosamine) after its partial N- deacetylation and hydrolysis [3, 6-9]. Chitin may be obtained from arthropods/exoskeletons of crustaceans such as crabs, shrimps, lobsters, fungi cell wall and insect shell (scorpions, spiders, cockroaches and silk worms) [10,11]. Chitosan has primary and secondary hydroxyl group along with free amino group. Chemical structure of chitosan is shown in Fig. (**1**).

3. CHITOSAN AND DRUG DELIVERY

Chitosan represents many unique properties and wide range of applications in the field of pharmaceutics, genetics and biotechnology (as shown below) [1]. From the past few years chitosan has become first choice of scientific community in delivering drugs as well as genes to the target site [12]. Favorable properties of chitosan are enlisted in Table **1** and shown in Fig. (**2**). Such properties make chitosan suitable for the development of ocular formulations like lenses, gels, inserts, colloidal systems etc.

4. PHYSICO-CHEMICAL PROPERTIES OF CHITOSAN

4.1. Solubility

Despite so many applications and unique properties, chitosan faces some limitations in its solubility depending upon its molecular structure and pKa. Chitosan and chitin has been defined and differentiated by their different solubility profile. Chitin is insoluble in organic solvents whereas chitosan has limited solubility, such as it is soluble in all acidic organic solvents like acetic acid (1-3%), tartaric acid and citric acid (4%) at pH less than 6.5 [22]. This limited solubility of chitosan is due to its highly crystalline structure which en-

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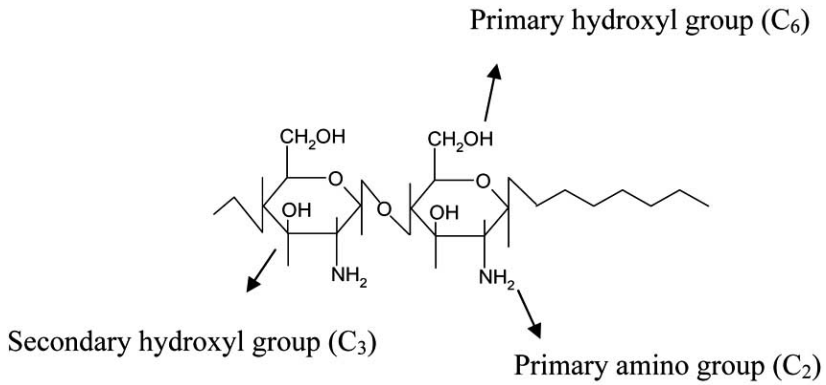

Fig. (1). Chemical structure of chitosan showing its important functional groups.

hanced its inter and intra molecular hydrogen bonding. However tailoring of chitosan by its chemical modifications and derivatization make it soluble in wide range of pH conditions. Chitosan shows a strong positive charge, cationic character when added in acidic solutions due to the presence of primary amine group on its backbone as shown in Fig. (**3**). These positively charged groups interact with its counter ion (drugs, excipients, ions and polymers). Chung *et al.* in 2006 studied the solubility of chitosan via Maillard reaction and examined its rheological and physico-chemical properties. They found that modified derivative of chitosan exhibits good solubility profile as compared to its native form [23].

4.2. Molecular Weight (MW)and Degree of Deacetylation (DD)

MW of chitosan is an important parameter to be considered during formulation development as it interferes with barrier properties and mechanical strength of polymer. Felt and coworkers in 2001 determined the effect of MW, DD and concentration of chitosan on different pharmacokinetic parameters (AUC_{eff}, t_{eff}) of antibiotics delivered to the eye. They suggested that chitosan when used as a carrier to transport tobramycin and ofloxacin in the tear film improved their AUC_{eff} and t_{eff} by enhancing their precorneal residence time [24]. Park *et al.* in 2002 investigated the effect of different MW chitosan and type of solvent on the properties of chitosan films [25]. Tensile strength of film increased with chitosan molecular weight. Acetic acid resulted in the toughest films followed by malic, lactic, and citric acid, respectively. Water vapor permeability was not influenced significantly by the molecular weight of chitosan. Oxygen permeability of films prepared with malic acid was the lowest, followed by acetic, lactic, and citric acid. Tang *et al.* in 2003 studied the effect of ultrasonication on the MW and DD of chitosan and observed that with increase in the duration and amplitude of ultrasonication MW and DD of chitosan nanoparticles decreases [26]. In another study Huang *et al.* in 2005 suggested that MW and DD of chitosan have great effect on its transfection efficiency and uptake capacity when used in gene therapy. They found that chitosan having low MW and low DD (46%) showed lowest transfection efficiency and less capacity of uptake as compared to those having highest MW and high DD (88%) [27]. Agnihotri and Aminabhavi in 2007 studied the effect of cross linking agent and MW of chitosan on parameters such as size, entrapment efficiency and release rate of drug and found that mean size range of timolol maleate loaded chitosan nanoparticles was between 118 and 203nm with a zeta potential ranged from + 17 to +23, having an entrapment efficiency between 47.6 and 63.0 % when prepared by desolvation method while *in-vitro* studies in PBS (pH 7.4) showed slow release of timolol maleate [28].

4.3. Mucoadhesive Character

Chitosan, having a polycationic surface when interacts with mucin layer containing residues of sialic acid (negative charge), results in the development of molecular attractive forces that helps in generation of mucoadhesive effect [6] as shown in Fig. (**4**). Electrostatic interactions which are developed due to opposite charges of polymer and epithelial cells are responsible for the prolonged retention of ocular drugs in the target area. Mucoadhesive character of chitosan is very important from the delivery point of view in ocular tissues as

Table 1. List of Chemical, Physical and Biological Properties of Chitosan

Property	Reference
Inexpensive and abundant availability in nature	[2]
Non-toxicity	[2]
Biodegradable & biocompatiblity	[13, 14]
Film forming capacity	[2]
Viscoelastic nature	[2]
Antimicrobial activity	[15]
Pharmaceutical excipient	[16]
Non-allergic and wound healing ability	[17]
Widening the tight junctions of membrane	[18]
Enhancing the drug residence time	[3]
Muco-adhesiveness	[19]
Immuno-stimulating activity	[20]
Improving the bioavailability and absorption of drugs at the target site	[21]
Serves as permeation and absorption enhancer	[21]

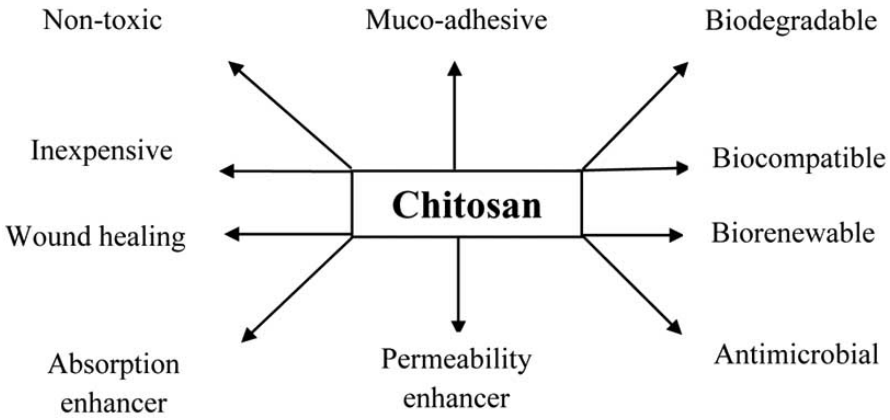

Fig. (2). Properties of chitosan that make it pharmaceutically acceptable biomaterial.

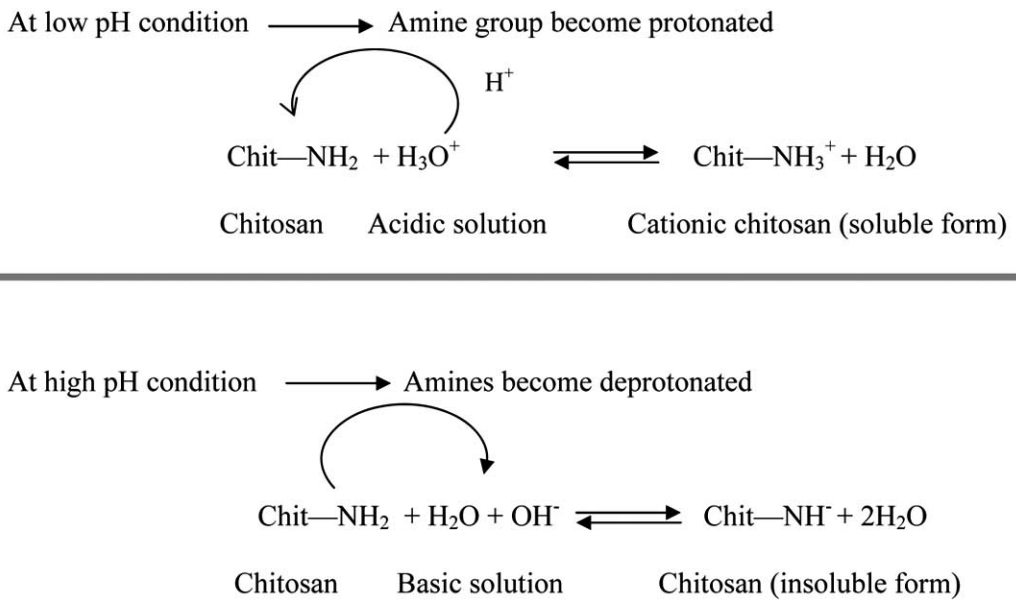

Fig. (3). Scheme of behavior of chitosan in various pH conditions.

primitive delivery systems such as ocular solutions and suspensions are not so much reliable and show very low ocular bioavailability and retention time when instilled. Chitosan fulfills a need for mucoadhesive biomaterial for ocular dosage.

4.4. Modification and Derivatization

Chitosan can be modified and derivatized to its quaternized form such as N-TMC and N- carboxymethyl chitosan. Earlier reports suggested that functional groups (presence of hydroxyl groups and amine groups) of chitosan can be readily modified to generate its improved derivatives. Modifications may be carried out generally due to following reasons:-

1) To improve the muco-adhesion of chitosan.
2) To open the tight junctions of epithelial cells.
3) To add some improved properties.
4) To enhance the penetration of drugs to the cornea [29].
5) To improve the solubility of chitosan [6, 30].

Di Colo G *et al.* in 2004 examined the in-vitro and in-vivo effect of three different derivatives of trimethyl chitosan (TMC) on the rate of drug permeation and found that permeation of ofloxacin was not dependent upon the MW of TMC's and its derivatives while in-vivo studies found increase in the trans-corneal permeation of ofloxacin when used for the treatment of endophthalmitis [31]. Recently Jayakumar *et al.* in 2008 focussed on the preparation of phosphorylated chitosan and chitin by different methods as these phosphorylated chitosan would become more biocompatible and helps in regeneration of tissues [30].

5. IMPORTANCE OF CHITOSAN IN OCULAR DRUG DELIVERY

Chitosan is a linear bifunctional polymer which enhanced ocular drug delivery due to its mucoadhesive effect. Ocular tissues such as cornea and conjunctiva possess anionic charge so their interaction with cationic mucoadhesive polymers might prolong the residence time of drug in the tear film and improved the patient compliance [32]. From the last few years several ocular formulations of chitosan were explored such as gels [3], implants, inserts [29], micro parti-

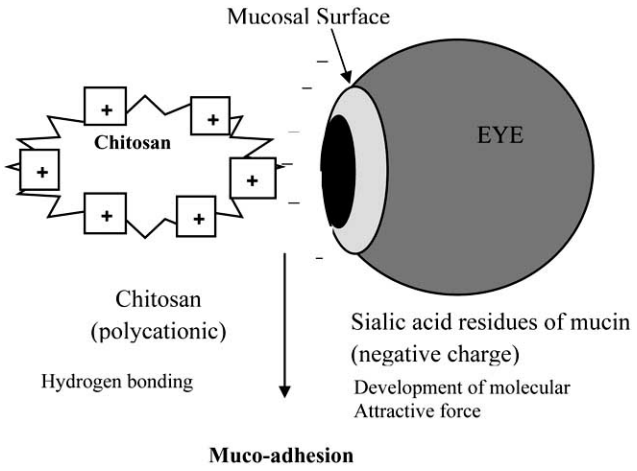

Fig. (4). Schematic presentation of mucoadhesive nature of chitosan due to strong ionic interactions.

cles, chitosan coated vesicular system [33] and nanoparticles (Fig. **5**). Drug entrapped in liposome and niosome colloidal systems improved the patient compliance and ocular bioavailability. It was further investigated that the retention time of drug was extended by coating these liposome /niosome via mucoadhesive polymer such as chitosan. The vesicles coated with chitosan extensively lowered the drug release rate with enhanced corneal residence time. Chenite *et al.* in 2001 proposed a novel approach by combining thermally sensitive neutral solution of chitosan/polyol salts [34]. These combinations remain liquid below room temperature but become gels at body temperature, so when injected in the cul-de-sac of eye it turns into a gel implants in-situ. De Campos *et al.* in 2001, demonstrated that cyclosporin A loaded chitosan nanoparticles formulated by ionic gelation technique enhanced the therapeutic level of drug when instilled in ocular tissues as compared to aqueous solution of cyclosorin A and showed faster release of drug during the first hour followed by a slow gradual release [32]. De Com-

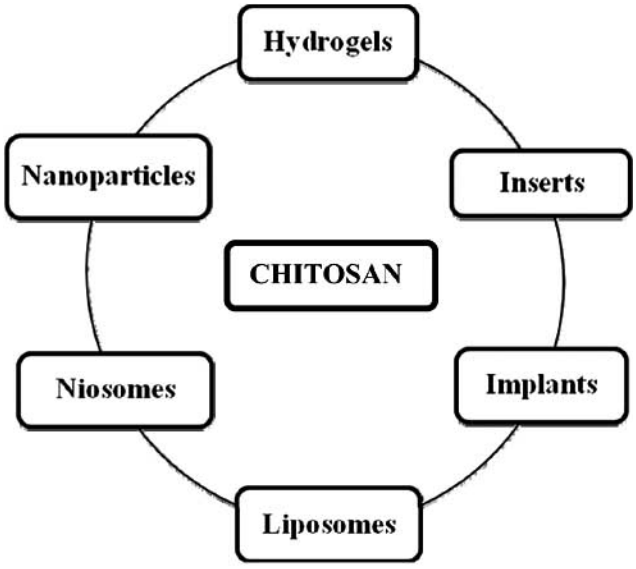

Fig. (5). Chitosan used in fabrication of different pharmaceutical formulation.

pos *et al.* 2004 investigated chitosan based nanoparticles for ocular routes and observed that fluorescent encapsulated chitosan nanoreservoir system was stable when incubated with lysozyme while high amount of chitosan nanoparticles was found in corneal tissues and conjunctiva as compared to chitosan solutions [35]. Ludwig 2005 has written an excellent review on mucoadhesive polymers. It is discussed that mucoadhesive effect of chitosan may be affected by the pH of the ocular environment as the alkaline /neutral pH of tears increased the muco-adhesive performance of chitosan [6]. Diebold *et al.* 2007 demonstrated that complexes of liposome-chitosan nanoparticles may be promising drug delivery systems in ocular therapy [36]. They showed negeligible toxicity when distributed on cultures of conjunctival epithelium and *in-vivo* studies revealed that these complexes are safe to use and are well tolerated. Chitosan (polycationic) interacts with polyanionic polymers to form a complex via ionotropic gelation and then can be used as a drug/gene carrier. Motwani *et al.* 2008 formulated chitosan-sodium alginate nanoparticles loaded with gatifloxacin by ionic gelation or modified coacervation technique to prolong the release of drug at the ocular surface and observed that the drug was released in a sustained fashion by non-fickian diffusion [37]. Modified chitosan nanoparticulate system was proposed for macromolecules such as genetic materials, peptides, proteins and other biotechnological products by De la fuente 2008. Chitosan-hyaluronan based nanocarriers showed better ocular bioavailability of bovine serum albumin (BSA) & cyclosporine A (CyA) [38]. Rodrigues *et al.* in 2008 suggested the use of monolayer/bilayer films of chitosan as a promising drug delivery carrier for the treatment of ocular disorders [8]. These studies showed that 89.6% of dexamethasone was released from the monolayer film of chitosan in the first 8 hr, while the bilayer film of chitosan released about 84% in 4 weeks. Moreover earlier studies revealed that among all the controlled release systems and colloidal carriers, priority was given to chitosan nanoparticles for the delivery of ocular therapeutics. A list of studies of chitosan formulation in ocular drug delivery and gene therapy are provided in Table **2** and Table **3** respectively.

6. UPTAKE AND TOXICITY OF CHITOSAN BASED OCULAR FORMULATIONS

6.1. Uptake of Nanoparticles in Ocular Tissues

Chitosan nanoparticles are internalized in corneal epithelium for the treatment of ocular disorders via endocytosis. De la fuente *et al.* in 2008 observed that when these chitosan nanoparticles instilled topically on the ocular surface, their uptake takes place in corneal and conjunctival cells as they get entered in the epithelial layer and assimilated by them. Chitosan nanoparticles provide high amount of drug to the target site by extending its attachment at the ocular surface and get distributed inside the ocular tissues via transport through the transcellular mechanism [38]. Further studies revealed that chitosan nanoparticles remain attached to the ocular mucosa for a longer period of time when combined with hyaluronic acid as both biopolymers are mucoadhesive in nature and prolonged the corneal residence time. Hyaluronic acid has mucoadhesion property through receptor mediated binding (CD44) which favours the interaction of chitosan- hyaluronan nanoparticulate system with the ocular

Table 2. List of Work Carried out Using Chitosan as Ophthalmic Carrier and/or for Surface Modification of Carrier

Formulation	Drug	Methods	Results	References
Insert- microspheres	Ofloxacin	Spray drying	Studies indicated that Chitosan micro particles in addition to PEO inserts prolonged the release of ofloxacin and enhanced its transcorneal permeation	[29]
Nanoparticles	Cyclosporin A	Modified ionic gelation	In-vitro studies revealed the fast release of CyA loaded nanoparticles (293 nm) during the first hour followed by a more gradual released of drug during a 24 hr period. Chitosan enhanced the therapeutic index of drug and in-vivo studies observed, high therapeutic concentration in external ocular tissues after its topical instillation	[32]
Nanoparticles	Fluorescent	Ionic gelation	*In-vitro* studies showed that chitosan fluorescent (Cs-fl) nanoparticles was stable upon incubated with lysozyme & confocal studies showed high amount of (Cs-fl) NP in corneal tissues as compared to Cs-fl solutions with 100% cell viability	[35]
Niosomes	Timolol	Reverse phase evaporation	In-vitro studies showed that timolol entrapped in niosomal delivery system and coated with chitosan extended the drug released and help in lowering of IOP with minimum side effects	[33]
Nanoparticles	Fluorescein isothiocyanate- BSA	Ionotropic gelation	Alonso observed that nanoparticles formulated by ionic gelation technique are well tolerated and safe to use in ocular drug delivery. These chitosan nanoparticles were internalized by IOBA-NHC cell cultures via active transport mechanism and showed high viability	[40]
Inserts	Pilocarpine	Reaction of acrylic acid-functionalized chitosan	In-vivo studies indicated that these chitosan/ hybrid systems are potential drug delivery systems for the treatment of ocular disorders	[41]
Nanoparticles	Gatifloxacin	Modified coacervation	In-vitro studies showed fast release of gatifloxacin nanoparticles during the first hr followed by a more gradual release during a 24 hr period	[37]
Nanoparticles Nanoemulsions	Indomethacin	Modified ionic gelation	Studies showed that high level of drug was found *in-vivo* as compared to solutions. Chitosan prolonged the delivery of indomethacin and enhanced its bioavailability in both external & internal ocular tissues	[42]
Nanoparticles	Dorzolamide	Ionic gelation	*In-vitro* studies showed that dorzolamide was released in a sustained manner in PBS (pH 7.4) when delivered in ocular tissues for the treatment of glaucoma	[43]
Nanoparticles	Gene delivery (GFP,RFP)	Coacervation	PCEP & MNP nanoparticles are nontoxic and shows high transfection efficiency while MNP yields good transfection as compared to PCEP	[9]
Nanoparticles	Gatifloxacin sesquihydrate	Solvent casting	Drug was released in a sustained manner and showed good antimicrobial efficiency	[44]

Table 3. Chitosan Nanoparticles in Ocular Gene Therapy

Genes	Vector	Outcomes	References
Plasmid (pEGFP) (p-beta-gal)	Non-viral (Hyaluronic acid-chitosan nanoparticles)	Chitosan- hyaluronic acid nanoparticles successfully encapsulated genes for the treatment of ocular disorders and showed high level of transfection capacity without affecting the viability of cells	[39]
DNA	Non-viral (Hyaluronic acid-chitosan nanoparticles)	Hyaluronic acid-chitosan nanoparticles loaded with DNA showed high transfection efficiency and were effective even when used in-vivo	[38]

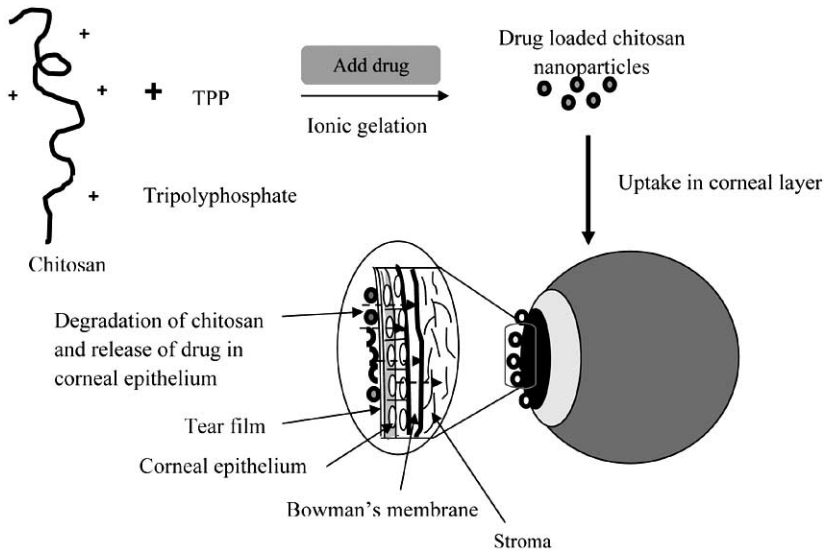

Fig. (6). Scheme of development of chitosan nanoparticles by ionic gelation method and their fate within ocular tissues.

epithelium. Chitosan-hyaluronic acid nanoparticles formulated via mild ionotropic gelation are promising delivery systems for the transfer of genes to the ocular tissues with high transfection efficacy as they are internalized via fluid endocytosis through CD44 receptor [39]. Transcellular transport occurs in the corneal endothelium via absorptive endocytic pathways and corneal endothelium plays an active role for the transfer of drugs and nutrients across the stroma [45]. Fig. (**6**) shows schematically the fate of chitosan nanoparticles on contact with ocular tissue.

6.2. Toxicity Profile of Chitosan

Chitosan is an environment friendly polyelectrolyte and exhibit a very low toxicity profile but the level of toxicity depends mainly upon its chemical composition. Chitosan has shown good tolerance in the ocular tissues, its LD_{50} is 16g/kg body weight of mice when given orally [46]. Table **4** summarizes the studies which have been carried out to evaluate the toxicity of chitosan in ocular tissues. Felt *et al.* in 1998 reported that low MW of chitosan in low concentration is safe to use and are well tolerated as compared to high MW

Table 4. List of Work Carried out with Toxicological Evaluation of Chitosan and its Derivative in Ophthalmics

Formulation	Evaluation	Remarks	References
Gel	Tolerance studies	Chitosan exhibited excellent tolerance on the corneal surface & 3 fold increase in corneal residence time but irritation occurs at high concentration (1.5%) & high MW of chitosan	[3]
Nanoparticles	Interaction with ocular surface	Chitosan in the form of nanoparticles helps in improving their interaction with the ocular surface and minimizing the systemic absorption	[32]
Nanoparticles	*In-vivo* toxicity (conjunctival cells)	Studies showed that no toxicity was observed in high concentration(2 mg/ml) and cells were fully viable but when the concentration was increased (>2 mg/ml) chitosan damaged the conjunctival cells	[35]
Liposome-chitosan-nanoparticles (LCs-NP)	*In-vitro* & *in-vivo* Toxicity	Studies revealed that chitosan nanoparticles showed lower cell viability than LCs-NP but exhibits negeligible toxicity *in-vitro* and good tolerance *in-vivo*	[36]
Nanoparticles	Toxicity & *in-vivo* tolerance	Chitosan showed no sign of discomfort at 24 hrs but the treated eye showed mild irritation due to the aggregation of chitosan nanoparticles in the form of mucus like discharge	[40]
Nanoparticles	*In-vitro* studies	Chitosan interacted with the extra ocular structure of eye and increased the residence time of drug. Due to reduced dosing frequency chitosan nanoparticles would provides better patient compliance	[37]
In-situ gel (thermoresponsive)	Toxicity	Studies showed that poly(N isopropylacryl amide)- chitosan in-situ gel forming system exhibited little cytotoxicity at the concentration range of 0.5-400µg/ml when determined by MTT assay	[47]
Nanoparticles	*In-vivo* toxicity (retina)	Chitosan is a natural non-toxic polymer but its contaminants (lipopolysaccharides) can induce inflammation in the vitreous chamber when injected in the eye	[9]

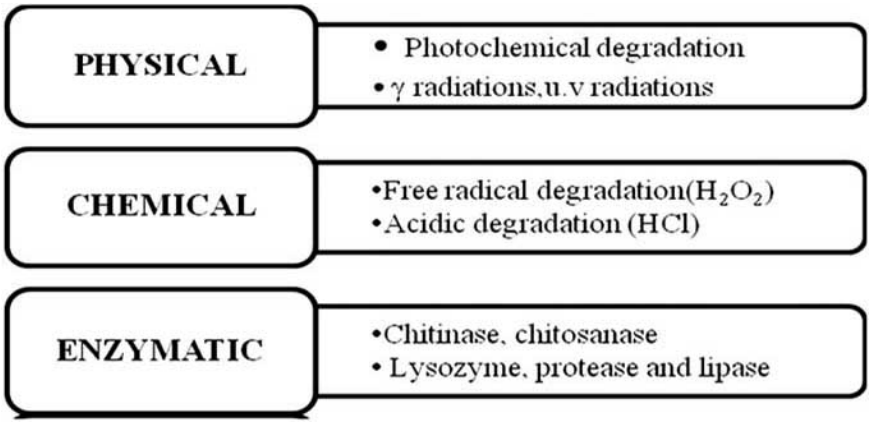

Fig. (7). Possible means and mechanism of degradation of chitosan.

chitosan [3]. They investigated this effect by studying the % of corneal lesions occurred by high MW chitosan (1930 kDa) at high concentration (1.5%) when delivered in the form of a gel [3]. De compos *et al.* in 2004 studied the *in-vivo* toxicity of chitosan nanoparticles in the conjunctival cells and observed no sign of toxicity *in-vivo* even at high concentration (2mg/ml), assessed by the trypan blue dye exclusion test. The 96% of the cells were viable in 2 mg/ml concentration in acetate buffer while the layer of conjunctival cells get damaged when the concentration of chitosan was increased up to 2mg/ml [35]. De Campos and co-workers addressed deeply about the chitosan nanoparticles on the basis of work done in the field of ocular drug delivery using chitosan. They suggested that chitosan nanoparticles are safe to deliver ocular bioactives [35]. Chitosan is biodegradable and broken down to harmless products and exhibited excellent tolerance in ophthalamics. Diebold *et al.* in 2006 studied the toxicity of liposome- chitosan nanoparticle complex both *in-vitro* and *in-vivo* in ocular tissues [36]. They found that concentration of chitosan is controlling factor of its toxicity to corneal and conjunctival cells.

6.3. Degradation and Fate of Chitosan

Chitosan is a high MW natural compound and prone to degradation by enzymes and hydrolysis. It is degraded by naturally occurring enzymes to small oligosaccharides and low MW compounds. Three common types of degradation pattern of chitosan are physical, chemical and enzymatic degradation as shown in Fig. (**7**). Breakdown products of chitosan are harmless and easily removed from the body. Fate of chitosan in ocular tissues is dependent upon ocular solutions (tear fluid), pH of ocular environment, presence of free radicals and ocular enzymes such as lysozyme etc. Tomihata and Ikade in 1997 studied the degradation behavior of chitosan both *in-vitro* and *in-vivo*. The *in-vitro* degradation rate was estimated by incubated them in a buffer solution (pH 7) having the enzyme lysozyme at 37^{o}C. The *in-vivo* studies showed very mild reaction as no sign of toxicity occurs [48]. Earlier reports showed that chitosan is commonly degraded to small chitooligomers by hydroxyl radicals which were decomposed from hydrogen peroxide and lead to chain scission. Agnihotri *et al.* in 2006 studied the effect of chemical modified form and concentration of acetic acid in solution on the degradation rate of chitosan. They observed that chitosan was stable in acetic acid solution without any degrading agent for about sixteen days and chemically modified form of chitosan was found more stable than chitosan when immersed with degrading agent at 37ºC [49]. Freier *et al.* in 2005 suggested that N-acetylation of chitosan affect its degradation pattern [50]. Chang *et al.* 2001 studied the kinetics of chitosan degradation products which was randomly degraded by hydrogen peroxide. It was observed that chemical degradation of chitosan was faster than its physical degradation. At high temperature (80ºC), chain scission of chitosan occurs and it degrades to small oligosaccharides [51]. Kang *et al.* in 2007 studied the synergistic effect of degradation agents (hydrogen peroxide and gamma radiation) on the fate of chitosan as observed by spectroscopic studies [52]. They reported that the crystallinity of chitosan decreases with degradation, and the crystalline state of water-soluble chitosan is entirely different from that of water-insoluble chitosan.

7. CONCLUSION

Chitosan is a versatile biofunctional polymer which controlled the rate of drug administered and its release fashion. It prolongs the residence time of drug and enhances its therapeutic effect. Chitosan possesses excellent physical, chemical and biological properties and shows diversified applications. Great deal of efforts has been taken by a lot of scientists and researchers worldwide to exploit the applications of chitosan in the field of ophthalmics. A better understanding of type of chitosan, derivatization and surface engineering may lead to successful ophthalmic product.

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ABBREVIATIONS

MW = Molecular weight

DD = Degree of deacetylation

TMC = Trimethyl chitosan

BSA = Bovine serum albumin

CyA = Cyclosporine A

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