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A Comprehensive Article

Ocular Pharmaceutical Drug Delivery System; A significant Roles with Properties, Preparations and Applications of Chitosan "Cationic Emulsifier "and Chitosan Nanoparticles Based ocular Delivery

Riad K. El Qidra
Department of Pharmaceutics and Industrial Pharmacy, College of Pharmacy, Al-Azhar University of Gaza, Gaza Strip, Palestine.

ABSTRACT : Amongst the various routes of drug delivery, the field of ocular drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. As an isolated organ, eye is very difficult to study from a drug delivery point of view. Due to various types of barriers such as different layers of cornea, sclera and retina including blood aqueous and blood–retinal barriers, choroidal and conjunctival blood flow etc. These barriers cause a significant challenge for delivery of a drug alone or in a dosage form, especially to the posterior segment of the eye. Despite these limitation, improvements have been made with the objective of maintaining the drug in the bio phase for an extended period and to overcome these problems, the newly developed particulate (nanoparticles) and vesicular systems like liposomes are useful in delivering the drug for a longer extent and helpful in reaching the systemic circulation hence improve their bioavailability. Polymeric nanoparticles have long been sought after as carriers for systemic and ocular drug delivery. Chitosan is biopolymers having immense structural possibilities for chemical and mechanical modifications to generate novel properties, functions and applications especially in ocular area. Chitosan is effective material for ocular applications because of their polyelectrolyte with reactive functional groups, gel-forming capability, high adsorption capacity, biocompatibility, biodegradability and non-toxicto living tissues as well as having antimicrobial activity, antifungal, antitumor activity and low immunogenicity, which clearly points to an immense potential for future development. These candidate biopolymers can be easily processed into gels, sponges, membranes, beads and scaffolds forms. Taking this information into account, the purpose of this review also to provide the reader with emphasizes recent research on different aspects of chitosan based ocular drug delivery including the number of applications of chitosan in eye disorder.

Key word: Ocular drug delivery, surfactants, chitosan, chitosan nanoparticles,

NTRODUCTION

The eye-ball is an organ protected from exogenous substances and external stress by various barriers figure [(1)], therefore, therapeutic drugs must be transported across several protective barriers regardless of which administration route is utilized. Usually topical ocular eye-drops are used. An eye-drop, irrespective of the instilled volume, often eliminates rapidly within five to six minutes after administration, and only a small amount (1–3%) of an eye-drop actually reaches the intraocular tissue[(1)]. Thus, it is difficult to provide and maintain an adequate concentration of drug in the precorneal area. More than 75% of applied ophthalmic solution is lost via nasolachrymal drainage and absorbed systemically via conjunctiva, hence ocular drug availability is very low [(2)].To increase ocular bioavailability and prolong the retention time on the ocular surface, numerous ophthalmic vehicles such as viscous solutions, suspensions, emulsions, ointments, aqueous gels, and polymeric inserts, have been investigated for topical application to the eye.

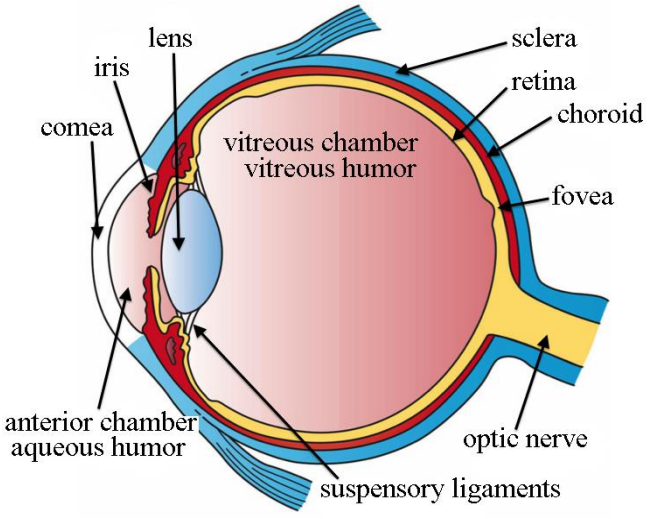


Figure (1): Schematic of the eye-ball structure.

Compliance is also problematic, particularly among patients who have chronic diseases such as glaucoma and refractory chorioretinal diseases, including uveitis, macular edema, neovascular (wet) and atrophic (dry) age-related macular degeneration (AMD), and retinitis pigmentosa (RP). It has been reported nearly 50% of glaucoma patients discontinued all topical ocular hypotensive therapy within six months. In addition, frequent intravitreal injections might cause complications, such as endophthalmitis and retinal detachment. Therefore, drug delivery systems(DDSs) for increasing patient's and doctor's convenience are also urgently needed.

2. Barriers to Restrict Intraocular Drug Transport :

Tear

One of the precorneal barriers is tear film which reduces the effective concentration of the administrated drugs due to dilution by the tear turnover (approximately 1 µL/min), accelerated clearance, and binding of the drug molecule to the tear proteins. In addition the dosing volume of instillation is usually 20–50 uL whereas the size of cul-de-sac is only 7–10 uL. The excess volume may spill out on the cheek or exit through the nasolacrimal duct [3-5]. For details of structure and function of tear film see[6].

Cornea

The cornea consists of three layers; epithelium, stroma and endothelium, and a mechanical barrier to inhibit transport of exogenous substances into the eye[7](figure 2). Each layer possesses a different polarity and a rate-limiting structure for drug permeation. The corneal epithelium is of a lipophilic nature, and tight junctions among cells are formed to restrict paracellular drug permeation from the tear film. The stroma is composed of an extracellular matrix of a lamellar arrangement of collagen fibrils. The highly hydrated structure of the stroma acts as a barrier to permeation of lipophilic drug molecules. Corneal endothelium is the innermost monolayer of hexagonal-shaped cells, and acts as a separating barrier between the stroma and aqueous humor. The endothelial junctions are leaky and facilitate the passage of macromolecules between the aqueous humor and stroma[8].

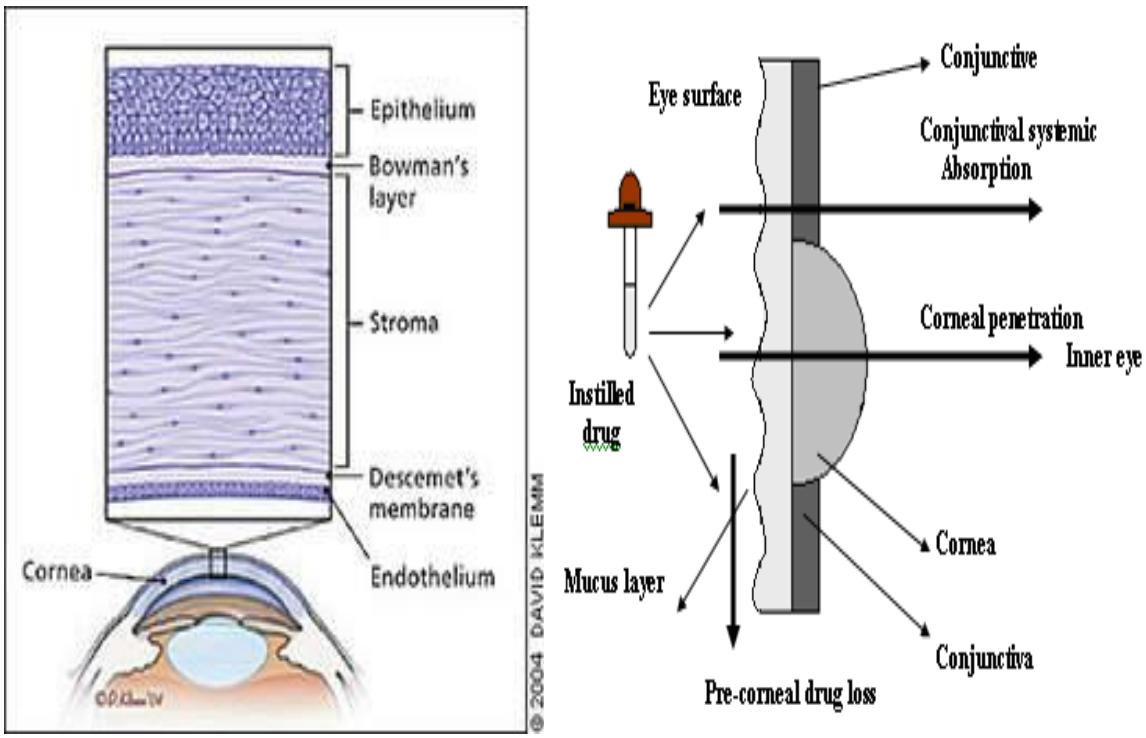


Figure (2): Schematic of corneal structure, its cellular organ of various transport-limiting barriers and transport pathways of a drug applied topically onto the eye.

Conjunctiva

Conjunctiva of the eyelids and globe is a thin and transparent membrane, which is involved in the formation and maintenance of the tear film. In addition, conjunctiva or episclera has a rich supply of capillaries and lymphatics[9-11], therefore, administrated drugs in the conjunctival or episcleral space may be cleared through blood and lymph. The conjunctival blood

vessels do not form a tight junction barrier[12], which means drug molecules can enter into the blood circulation by pinocytosis and/or convective transport through paracellular pores in the vascular endothelial layer. The conjunctival lymphatics act as an efflux system for the efficient elimination from the conjunctival space. Recently, it has been reported that at least 10% of a small molecular weight hydrophilic model compound (sodium fluorescein), administered in the subconjunctival space, is eliminated via the lymphatic's within the first hour in rat eyes[13]. Therefore, drugs transported by lymphatic's in conjunction with the elimination by blood circulation can contribute to systemic exposure, since the interstitial fluid is returned to the systemic circulation after filtration through lymph nodes.

Sclera

The sclera mainly consists of collagen fibers and proteoglycans embedded in an extracellular matrix[14]. Scleral permeability has been shown to have a strong dependence on the molecular radius; scleral permeability decreases roughly exponentially with molecular radius. Additionally, the posterior sclera is composed of a looser weave of collagen fibers than the anterior sclera, and the human sclera is relatively thick near the limbus (0.53 ± 0.14 mm), thin at the equator (0.39 ± 0.17 mm),and much thicker near the optic nerve (0.9–1.0 mm). Thus, the ideal location for transscleral drug delivery is near the equator at 12–17 mm posterior to the corneoscleral limbus. Hydrophobicity of drugs affects scleral permeability; increase of lipophilicity shows lower permeability; and hydrophilic drugs may diffuse through the aqueous medium of proteoglycans in the fiber matrix pores more easily than lipophilic drugs[15,16]. Furthermore, the charge of the drug molecule also affects its permeability across the sclera. Positively charged compounds may exhibit poor permeability due to their binding to the negatively charged proteoglycan matrix[17].

Choroid/Bruch's Membrane

Choroid is one of the most highly vascularized tissues of the body to supply the blood to the retina. Its blood flow per unit tissue weight is ten-fold higher than in the brain. In addition the choroidal capillary endothelial cells are fenestrated and, in humans, are relatively large in diameter (20–40 um).An Optical Coherence Tomography (OCT) can noninvasively measure the thickness of retina and choroid[18,19]. Using an OCT, it has been shown, choroidal thickness becomes thinner with age[20]. Previous histological studies have shown choroidal thickness changes from 200 μm at birth decreasing to about 80 μm by age 90 [21]. In addition, chorioretinal diseases including AMDwith pigment epithelial detachment, central serous chorioretinopathy, age-related choroidal atrophy[21], and high myopia[22], affect choroidal thickness. In contrast, Bruch's membrane (BM) causes thickening with age. These changes cause increased calcification of elastic fibers, increased cross-linkage of collagen fibers and increased turnover of glycosaminoglycans.

In addition, advanced glycation end products and lipofuscin accumulate in BM[23,24]. Thickness changes of choroid and BM might affect drug permeability from subconjunctiva or episcleral space into the retina and the vitreous.

Retina

The drugs in the vitreous are eliminated by two main routes from anterior and posterior segments. All drugs are able to eliminate via the anterior route. This means drugs can diffuse across the vitreous to the posterior chamber and, thereafter, eliminate via aqueous turnover and uveal blood flow. Elimination via the posterior route takes place by permeation across the retina. One of the barriers restricting drug penetration from the vitreous to the retina is the internal limiting membrane (ILM). The ILM separates the retina and the vitreous, and is composed of 10 distinct extracellular matrix proteins[25]. Although a previous study using primates has suggested that molecules exceeding 100 kDa cannot cross the retinal layers into the subretinal space, it has been confirmed by immunohistochemical analysis, a full-length, humanized, anti-vascular endothelial growth factor (VEGF) monoclonal antibody (Bevacizumab, Avastin®), composed of 214 amino acids with a molecular weight of 149 kDa, injected into the vitreous cavity, can penetrate through the sensory retina into retinal pigment epitheliums (RPE), subretinal and choroidal space, in monkey and rabbit. In addition, nanometer-sized particles whose mean diameter is below 200 nm can penetrate across the sensory retina into RPE after intravitreal injection in rabbit[26,27].

In intact retina, theoretically, the drugs in the subretinal fluid could either be absorbed by the sensory retinal blood vessels or transported across the RPE, where it may be absorbed into the choroidal vessels or pass through the sclera. Drug transport across the RPE takes place both by transcellular and paracellular routes. The driving forces of outward transport of molecules from the subretinal spaces are hydrostatic and osmotic, and small molecules might transport through the

paracellular inter-RPE cellular clefts and by active transport through the transcellular route.

Blood-Retinal Barrier

Blood-retinal barrier (BRB) restricts drug transport from blood into the retina. BRB is composed of tight junctions of retinal capillary endothelial cells and RPE, called iBRB for the inner and oBRB for the outer BRB, respectively[28]. The function of iBRB is supported by Müller cells and astrocytes. The retinal capillary endothelial cells are not fenestrated and have a paucity of vesicles. The function of these endothelial vesicles has been described as endocytosis or transcytosis that may be receptor mediated or fluid phase requiring adenosine triphosphate[29]. A close spatial relationship exists between Müller cells and retinal capillary vessels to maintain the iBRB in the uptake of nutrients and in the disposal of metabolites under normal conditions[30]. Müller cells are known to support neuronal activity and maintain the proper functioning of the iBRB under normalconditions. They are involved in the control and homeostasis of K+ and other ions signaling molecules, and in the control of extracellular pH. Dysfunction of Müller cells may contribute to a breakdown of the iBRB in many pathological conditions, such as diabetes. Müller cells enhance the secretion of VEGF under hypoxic and inflammatory conditions[31]. In vitrostudy has shown that VEGF-induced occluding phosphorylation and ubiquitination causes trafficking of tight junction and leads to increased retinal vascular permeability.

The astrocytes originate from the optic nerve and migrate to the nerve fiber layer during development. They are closely associated with the retinal capillary vessels[32] and help to maintain the capillary integrity. Astrocytes are known to increase the barrier properties of the retinal vascular endothelium by enhancing the expression of the tight junction protein ZO-1 and modifying endothelial morphology[33].

Following systemic drug administration, drugs can easily enter into the choroid since choroid is highly vascularized tissue compared to retina. The chorio capillaris are fenestrated resulting in rapid equilibration of drug molecules present in the bloodstream with the extravascular space of the choroid. Therefore, oBRB (RPE) restricts further entry of drugs from the choroid into the retina. RPE is a monolayer of highly specialized hexagonal-shaped cells, located between the sensory retina and the choroid. The tight junctions of the RPE efficiently restrict intercellular permeation into the sensory retina**.**

Ocular Pharmacokinetics[34,35]

The drug pharmacokinetics from the eye follows the following paths:

- Transcorneal permeation from the lacrimalfluid into the anterior chamber.
- Non-corneal drug permeation across the conjunctiva and sclera into the anterior uvea.
- Drug distribution from the blood stream via blood-aqueous barrier into the anterior chamber.
- Elimination of drug from the anterior chamber by the aqueous humor turn over to the trabecular meshwork and sclemm'scanal.
- Drug elimination from the aqueous humor into the systemic circulation across theblood-aqueous barrier.
- Drug distribution from the blood into theposterior eye across the blood-retinabarrier.
- Intravitreal drug administration.
- Drug elimination from the vitreous viaposterior route across the blood-retinabarrier.
- Drug elimination from the vitreous viaanterior route to the posterior chamber.

Drug delivery[36]: is the method or process of administering pharmaceutical compound to achieve a therapeutic effect in humans or animals. Drug delivery technologies modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy, safety, as well as patient compliance and convenience.

Novel drug delivery: A novel drug delivery system is a system that offer multiple drug delivery solutions such as:

- Oral Drug Delivery Systems and Materials
- Parenteral and Implant Drug Delivery Systems
- Pulmonary and Nasal Drug Delivery
- Transmucosal Drug Delivery
- Transdermal and Topical Drug Delivery
- Delivery of Proteins and Peptides
- Drug Delivery Pipelines
- Drug Delivery Deals

Recent Advances and Challenges in Ocular Drug Delivery System[37]

Recent advances in topical drug delivery have been made that improve ocular drug contact time and drug delivery, including the development of ointments, gels, liposome formulations and various sustained and

controlled-release substrates, such as the Ocusert, collagen shields and hydrogellenses. The development of newer topical delivery systems using polymeric gels, colloidal systems and cyclodextrins will provide exciting new topical drug therapeutics.

Following characteristics are required to optimize ocular drug delivery system:

a- Good corneal penetration.

b- Prolong contact time with corneal tissue.

c- Simplicity of instillation for the patient.

d- Non irritative and comfortable form (viscous solution should not provoke lachrymal secretion and reflex blinking)

e- Appropriate rheological properties and concentrations of the viscous system.

Recent Formulation Approaches to Improve Ocular Bioavailability[38]

Various approaches that have been attempted to increase the bioavailability and the duration of therapeutic action of ocular drugs can be divided into two categories.

1- based on the use of the drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs.
2- maximizing corneal drug absorption and minimizing pre corneal drug loss.

The typical pulse entry type drug release behavior observed with ocular aqueous solutions (eye drops), suspensions, and ointments can be replaced by a more controlled, sustained, and continuous drug delivery, using a controlled release ocular drug delivery system. These systems can achieve therapeutic action with a smaller dose and a fewer systemic and ocular side effects. Such systems include implantable systems, ocuserts, collagen shields, but the limitations of these systems include poor patient compliance, need of surgery, and difficulty in self-insertion. Particulate drug delivery systems, like nanoparticles and microspheres, can also be used to improve the residence time of the drug. Upon administration to the eye.

The particles reside at the delivery site and the drug is released from the particles through diffusion, chemical reaction, polymer degradation, or ion-exchange mechanism. Smaller particles are better tolerated by the patients than larger particles and hence microspheres and nanoparticles represent very comfortable prolonged action ophthalmic drug delivery systems. However, some workers observed that nanoparticles consisting of poly damaged the corneal epithelium by disrupting the cell membrane. Capacity of some polymers to adhere to the mucin coat covering the conjunctiva and the corneal surfaces of the eye by a non-covalent bond has been exploited to provide an intimate contact between the drug and the absorbing tissue, which may result in high drug concentration in the local area and hence, drug flux through the absorbing tissue. Common disadvantage observed is that the adhesive often detaches itself from the rate controlling drug delivery device and causes premature release of drugs. Increasing the permeability of the corneal epithelial membrane can maximize the transport characteristics across the cornea. Penetration enhancers or the absorption promoters can thus be used to increase the permeability of cell membrane or loosen the tight junctions or both12. large numbers of enhancers, like actin filament inhibitors, surfactants, bile salts, chealators, and organic compounds, have been used. However, the unique characteristics and great sensitivity of the corneal conjunctival tissues impose great caution in the selection of enhancers with regard to consideration of their capacity to effect the integrity of the epithelial surfaces. There is evidence that penetration enhancers themselves can penetrate the eye and may, therefore, lead to unknown toxicological complications, e.g. benzalkonium chloride (BAC) was found to accumulate in the cornea for days.

EDTA was found to reach the iris-ciliary body in concentrations high enough to alter the permeability of the blood vessels in the tract indirectly accelerating drug removal from aqueous humor. Bile salts and surfactants were found to cause irritation of the eye and nasal mucosa. Liposomes (vesicular/colloidal systems) are a potentially useful ocular drug delivery system due to the simplicity of preparation and versatility in physical characteristics, but suffer from the disadvantage of instability (due to the hydrolysis of phospholipids normally used in their preparation), limited drug loading capacity, and technical difficulties in obtaining a sterile liposomal preparation. It has been reported that the stearylamine used to prepare positive liposomes was toxic to the cells and also appeared to be irritating to the eye[39]

Advantages of controlled ocular drug delivery systems[40]:

1. Increased accurate dosing. To overcome the side effects of pulsed dosing produced by conventional systems.
2. To provide sustained and controlled drug delivery.

3. To increase the ocular bioavailability of drug by increasing the corneal contact time. This can be achieved by effective adherence to corneal surface.
4. To provide targeting within the ocular globe so as to prevent the loss to other ocular tissues.
5. To circumvent the protective barriers like drainage, lacrimation and conjunctival absorption.
6. To provide comfort, better compliance to the patient and to improve therapeutic performance of drug.
7. To provide better housing of delivery system.

Mechanism of controlled sustained drug release into the eye[34]:

The corneal absorption represents the major mechanism of absorption for the most conventional ocular therapeutic entities.

The mechanism of controlled drug release into the eye is as follows:

A. Diffusion
B. Osmosis
C. Bio-erosion

A. Diffusion

In the Diffusion mechanism, the drug is released continuously at a controlled rate through the membrane into the tear fluid. If the insert is formed of a solid non-erodible body with pores and dispersed drug. The release of drug can take place via diffusion through the pores. Controlled release can be further regulated by gradual dissolution of solid dispersed drug within this matrix as a result of inward diffusion of aqueous solutions. In a soluble device, true dissolution occurs mainly through polymer swelling. In swelling-controlled devices, the active agent is homogeneously dispersed in a glassy polymer. Since glassy polymers are essentially drug-impermeable, no diffusion through the dry matrix occurs. When the insert is placed in the eye, water from the tear fluid begins to penetrate the matrix, then swelling and consequently polymer chain relaxation and drug diffusion take place. The dissolution of the matrix, which follows the swelling process, depends on polymer structure: linear amorphous polymers dissolve much faster than cross-linked or partially crystalline polymers. Release from these devices follows in general Fickian 'square root of time' kinetics; in some instances, however, known as case II transport, zero order kinetics has been observed.

B. Osmosis

In the Osmosis mechanism, the insert comprises a transverse impermeable elastic membrane dividing the interior of the insert into a first compartment and a second compartment; the first compartment is bounded by a semi-permeable membrane and the impermeable elastic membrane, and the second compartment is bounded by an impermeable material and the elastic membrane. There is a drug release aperture in the impermeable wall of the insert. The first compartment contains a solute which cannot pass through the semi-permeable membrane and the second compartment provides a reservoir for the drug which again is in liquid or gel form. When the insert is placed in the aqueous environment of the eye, water diffuses into the first compartment and stretches the elastic membrane to expand the first compartment and contract the second compartment so that the drug is forced through the drug release aperture.

C. Bioerosion

In the Bioerosion mechanism, the configuration of the body of the insert is constituted from a matrix of bio erodible material in which the drug is dispersed. Contact of the insert with tear fluid results in controlled sustained release of the drug by bio erosion of the matrix. The drug may be dispersed uniformly throughout the matrix but it is believed a more controlled release is obtained if the drug is superficially concentrated in the matrix.

In truly erodible or E-type devices, the rate of drug release is controlled by a chemical or enzymatic hydrolytic reaction that leads to polymer solubilization, or degradation to smaller, water-soluble molecules. These polymers, which as specified by Heller, may undergo bulk or surface hydrolysis. Erodible inserts undergoing surface hydrolysis can display zero order release kinetics; provided that the devices maintain a constant surface geometry and that the drug is poorly water-soluble.

Ophthalmic drug product may be classified according to route of administration.

1. Topical
2. Intraocular
3. Systemic (oral and venous).

Absorption of drugs in the eye takes place either through corneal or non-corneal route. Maximum absorption takes place though the cornea, which leads the drug into aqueous humor. Loss of the administered dose of drug, takes place through spillage and removal by the naso-lacrimal apparatus. The non corneal route involves the absorption across the sclera and conjunctiva into the intra ocular tissues.

CLASSIFICATION OF OCULAR DRUG DELIVERY SYSTEMS[41,42]:

A multitude of ocular dosage forms are available for delivery of drugs to the eye. These can be classified on the basis of their physical forms as follows:

1. Liquids: Solutions, Suspensions, Sol to gel systems, Sprays
2. Solids: Ocular inserts, Contact lenses, Corneal shield, Artificial tear inserts, Filter paper strips.
3. Semi-solids: Ointments, Gels.
4. Miscellaneous: Ocular iontophoresis, Vesicular systems, Muco-adhesive dosage forms, Particulates.

1. Liquids : Liquids are the most popular and desirable state of dosage forms used for the eye. This is because the drug absorption is fastest from this state. The slow release of the drug from the suspended solids provides a sustained effect for a short duration of time.

Solutions and Suspensions:

Solutions

Solutions are the pharmaceutical forms most widely used to administer drugs that must be active on the eye surface or in the eye after passage through the cornea or the conjunctiva. The drug in the solution is in the dissolved state and may be immediately active. This form also have disadvantages; the very short time the solution stays at the eye surface, its poor bioavailability (a major portion i.e. 75% is lost via nasolacrimal drainage), the instability of the dissolved drug, and the necessity of using preservatives. A considerable disadvantage of using eye drops is the rapid elimination of the solution and their poor bioavailability. This rapid elimination is due to solution state of the preparation and may be influenced by the composition of the solution. The retention of a solution in the eye is influenced by viscosity, hydrogen ion concentration, the osmolality and the instilled volume. Extensive work has been done to prolong ocular retention of drugs in the solution state by enhancing the viscosity or altering the pH of the solution.

Suspensions

Suspensions are called as dispersion of finely divided relatively insoluble drug substances in an aqueous vehicle which contains suitable amount of suspending and dispersing agents. Because of a tendency for the particle to be retained in the cul-de-sac, the contact time and duration of action of a suspension exceed those of a solution. While the retention increases with an increase in the particle size, so does the irritation of the eye. The rate of the dissolution of the suspended drugs increases with decreasing particle size. Thus an optimum particle size has to be selected for each type of drug, and it is recommended that the particles in an ophthalmic suspension should be not more than 10 µm in size.

Sol-Gel Systems

The new concept of producing a gel in situ (e.g. in the cul-de-sac of the eye) was suggested for the first time in the early 1980s. It is widely accepted that increasing the viscosity of a drug formulation in the precorneal region will lead to an increased bioavailability, due to slower drainage from the cornea. Several concepts for the in situ gelling systems have been investigated. These systems can be triggered by change in pH, temperature or by ion activation. An anionic polymeric dispersion shows a low viscosity up to pH 5. 0, and will coacervate in contact with tear fluid due to presence of a carbonic buffer system which regulates the pH of tears. In situ gelling by a temperature change is produced when the temperature of polymeric dispersion is raised from 25 to 37°C. Ion activation of polymeric dispersion occurred due to the presence of cations in the tear fluid. A solution containing 1.5% methyl cellulose and 0.3% carbopol at pH 4.0 and 25°C was found to be an easily flowing liquid capable of administration as a drop and showed an increase in viscosity and conversion to a gel on changing pH to 7.4 by addition of 0.5 M NaOH.

Sprays[43]

Although not commonly used, some practitioners use mydriatics or cycloplegics alone or in combination in the form of eye spray. These sprays are used in the eye for dilating the pupil or for cycloplegics examination.

2. Solids:

The concept of using solids for the eye is based on providing sustained release characteristics.

Ocular inserts

Ocular inserts are solid dosage form and can overcome the disadvantage reported with traditional ophthalmic systems like aqueous solutions, suspensions and ointments. The typical pulse entry type drug release behavior observed with ocular aqueous solutions (eye drops), suspensions and ointments is replaced by more controlled, sustained and continuous drug delivery using a controlled release ocular drug delivery system. The eye drops provided pulse entry pattern of drug administration in the eye which is characterized by transient overdose, relatively short period of acceptable dosing, followed by prolonged periods of under dosing. The ocular inserts maintain an effective drug concentration in the target tissues and yet minimize the number of applications consonant with the function of controlled release systems. Limited popularity of ocular inserts has been attributed to psychological factors,

such as reluctance of patients to abandon the traditional liquid and semisolid medications, and to occasional therapeutic failures (e.g. unnoticed expulsion from the eye, membrane ruptures etc.). A number of ocular inserts were prepared utilizing different techniques to make soluble, erodible, non-erodible, and hydrogel inserts[44,45].

Insoluble insert

These are solid or semisolid sterile preparations. Of appropriate size and shape, designed to be inserted behind the eyelid or held on the eye and to deliver drugs for topical or systemic effects these are polymeric systems into which the drug is incorporated as a solution or dispersion.

Ocular therapeutic system (OTS) or minidisc.

These are controlled – release monolithic matrix-type devices consisting of a contoured disc with a convex front and a concave back surface, designed so as to fit the eyeball. The OTS can be made hydrophilic or hydrophobic to permit extended release of both water-soluble and water-insoluble drugs. They were reported to be very comfortable when placed behind the top or bottom of the eyelid.

Contact lenses[46]

Contact lenses can absorb water soluble drugs when soaked in drug solutions. These drug saturated contact lenses are placed in the eye for releasing the drug for long period of time. The hydrophilic contact lenses can be used to prolong the ocular residence time of the drugs. In humans, the Bionite lens which was made from hydrophilic polymer (2-hydroxy ethyl methacrylate) has been shown to produce a greater penetration of fluorescein.

Corneal shield

Topically applied antibiotics have been used in conjunction with the shield to promote healing of corneal ulcers. Collagen shields are fabricated with foetal calf skin tissue and originally developed as a corneal bandage. These devices, once softened by the tear fluid, form a thin pliable film that confirms exactly to the corneal surface, and undergoes dissolution up to 10, 24 or 72 hours. Collagen film proved as a promising carrier for ophthalmic drug delivery system because of its biological inertness, structural stability and good biocompatibility. Gussler et al investigated the delivery of trifluoro thymidine (TFT) in collagen shields and in topical drops in the cornea of normal rabbits and corneas with experimental epithelial defects. It was found that highest drug concentrations were found in the eyes treated with shields as compared to eye drops[47].

Artificial tear inserts[48].

A rod shaped pellet of hydroxypropyl cellulose without preservative is commercially available (Lacrisert). This device is designed as a sustained release artificial tear for the treatment of dry eye disorders.

Filter paper strips

Sodium fluorescein and rose Bengal dyes are commercially available as drug impregnated filter paper strips. These dyes are used diagnostically to disclose corneal injuries and infections such as herpes simplex, and dry eye disorders.

3. Semi solids

A wide variety of semisolids vehicles are used for topical ocular delivery which falls into two general categories: simple and compound bases. Simple bases refer to a single continuous phase. These include white petrolatum, lanolin and viscous gels prepared from polymers such as PVA, carbopol etc. Compound bases are usually of a biphasic type forming either water in oil or oil in water emulsions. A drug in either a simple or compound base provide an increase in the duration of action due to reduction in dilution by tears, reduction in drainage by way of a sustained release effect, and prolonged corneal contact time. The most commonly used semisolid preparation is ointments consisting of dispersion of a solid drug in an appropriate vehicle base.

Semi-solids dosage forms are applied once or twice daily and provide sustained effects. The primary purpose of the ophthalmic ointment vehicle is to prolong drug contact time with the external ocular surface. But they present a disadvantage of causing blurring of vision and matting of eyelids. Ophthalmic gels are similar in viscosity and clinical usage to ophthalmic ointments. Semi-solids vehicles were found to prolong the ocular contact time of many drugs, which ultimately leads to an enhanced bioavailability[49].

4. Miscellaneous

Vesicular systems

Vesicular systems have been developed to provide improvement in ocular contact time, providing sustained effect and reducing side effects of the drug(s) entrapped.

Liposome's

Liposome's are phospholipids-lipid vesicles for targeting the drugs to the specific sites in the body. Because of their structural versatility they can

incorporate any kind of drug substance regardless of its solubility. They provide the controlled and selective drug delivery and improved bioavailability and their potential in ocular drug delivery appears greater for lipophilic than hydrophilic compounds. Liposome's are vesicles composed of a lipid membrane enclosing an aqueous volume. Liposome's offer the advantage of being completely biodegradable and relatively nontoxic but are less stable than particulate polymeric drug delivery systems. Liposome's were found to be potential delivery system for administration of a number of drugs to the eye[50,51].

Niosomes

In order to circumvent the limitations of liposome's as chemical instability, oxidative degradation of phospholipids, cost and purity of natural phospholipids, niosomes are developed as they are chemically stable as compared to liposome's and can entrap both hydrophilic and hydrophobic drugs. They are nontoxic and do not require special handling techniques.

Pharmacosomes[52]

This is the term used for pure drug vesicles formed by the amphiphillic drugs. Any drugs possessing a free carboxyl group or an active hydrogen atom (-OH, NH_2) can be esterified (with or without a spacer group) to the hydroxyl group of a lipid molecule, thus generating an amphiphillic prodrug. The amphiphillic prodrug is converted to pharmacosomes on dilution with water. The pharmacosomes show greater shelf stability, facilitated transport across the cornea, and a controlled release profile.

Muco-adhesive dosage form (Bioadhesive polymers):

This approach relies on vehicles containing polymers which will attach, via non- covalent bonds, to conjunctival mucin (a glycoprotein) thus remaining in contact with the precorneal tissues until mucin turnover cause elimination of the polymer. Muco-adhesive polymers are usually macromolecular hydrocolloids with numerous hydrophilic functional groups, such as carboxyl -, hydroxyl-, amide and sulphate capable of establishing electrostatic interactions. Hui and Robinson synthesized polymers of acrylic acid cross-linked with divinyl glycol and 2, 5 - dimethyl - 1, 5 - hexadiene and examined their utility in ocular drug delivery.

Selective targeting of drugs to the proposed site of action provides therapeutic advantages such as reduced toxicity and smaller dose levels. Bioadhesive systems which will utilize in- tense contact to increase the drug concentration gradient could be an attractive approach. Because of their specific carbohydrate-binding, lectins can interact with glycoconjugates present on the epithelial cells that line all of the organs exposed to the external environment. The unique carbohydrate specificities of plant lectins can facilitate mucoadhesion and cytoadhesion of drugs[53].

Particulates(Nanoparticles/Microparticles):

Particulate polymeric drug delivery systems include micro- and nanoparticles. Particles in the micrometer size range >1mm are called Microparticles or microspheres, whereas those in the nanometer size range < 1mm (1000 nm) are called nanoparticles. Microparticles with a capsule wall enclosing a liquid or solid core are called microcapsules. The upper size limit for Microparticles for ophthalmic administration is about 5-10 mm. above this size, a scratching feeling in the eye can result after ocular application. Microspheres and Nanoparticles represent promising drug carriers for ophthalmic application. The binding of the drug depends on the physicochemical properties of the drugs as well as of the nano or micro particle polymer. Particulates such as nanoparticles, nanocapsules, submicron emulsions, nanosuspensions improved the bioavailability of ocularly applied drugs[54-57].

Surfactants (polymers)[58]

Surfactants are macromolecules having very large chains, contain a variety of functional groups, can be blended with low and high molecular weight materials. Surfactants are becoming increasingly important in the field of drug delivery. Advances in surfactant science have led to the development of several novel drug delivery systems. A proper consideration of surface and bulk properties can aid in the designing of surfactants for various drug delivery applications. These newer technological development include drug modification by chemical means, career based drug delivery and drug entrapment in polymeric matrices or within pumps that are placed in desired bodily compartments. These technical development in drug delivery approaches improve human health. Use of polymeric material drug delivery approaches has attracted the scientists[59].

Furthermore, surfactants are frequently used as emulsifiers in formulations for Ocular application. A substance which is positively adsorbed at the liquid/vapour and/or at other interfaces is called surfactants. Surfactants are usually organic compounds

that are amphiphilic, meaning they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads). Therefore, a surfactant molecule contains both a water insoluble (and oil soluble) component and a water soluble component. Surfactant molecules will diffuse in water and adsorb at interfaces between air and water or at the interface between oil and water, in the case where water is mixed with oil[60].

Classification of surfactants:

Surfactants can be classified into four main categories according to the presence of formally charged groups in the head;

- anionic (e.g. sodium laurylsulfate),
- cationic (e.g. chitosan and cetyltrimethyl ammonium bromide),
- nonionic (e.g. polyoxyethylene sorbitan monopalmitate) and
- amphoteric (e.g. N-dodecyl-N, N-dimethylbetaine).

Mechanism of action of cationic surfactants (Charge Effect) as penetration enhancersfollowed by topical instillation:

Cationic surfactants

Attempts have been made to prolong the time of residence in ocular tissues followed by topical instillation by means of electrostatic adhesion of droplets over the corneal surface. It was initially believed and now has become clearer from many reports in the literature that an occurrence of electrostaticattraction between the cationic emulsified droplets and anionic cellular moieties of the ocular tissues exists[61]. As the corneal area is negatively charged (negative charges of sialic acid), the positively charged droplets might bind to the sites. The charge is provided by a positively charged lipid, for example, stearylamine or cationic polysaccharide, for example, chitosan and stearylamine.

Beilin and coworkers reported that the presence of positive charge on the surface of internal phase could influence drug absorption through corneal penetration[62]. The supposition was based on the presence of negative charge on the corneal surface which would facilitate binding of positively charged droplets of the submicron emulsion[63].

Calvo and coworkers studied comparative behavior of drug release through colloidal systems, namely, nanocapsules, nanoparticles, and submicrons emulsion, the findings of which showed an increased corneal permeation of indomethacin due to incorporation of the drug in colloidal carriers instead of the electrostatic attraction between the negatively charged cornea and positively charged drug carrier system. They found that the incorporation of the drug into a colloidal system facilitates the uptake of nanoglobules by the corneal epithelium without causing any damage to the cell membrane[64].

Chitosan

Chitosan (CS) is modified natural, biodegradable, biocompatible, non-toxic, as well as linear nitrogenous polysaccharides, a basic polysaccharide homo-polymer[65], which is the second-most abundant polymer in nature after cellulose. CS is produced commercially by deacetylation of chitin, naturally occurring polysaccharides which is the structural element in the exoskeleton of crustaceans such as shrimps, lobsters, and crabs, etc(figure 3).The shells of these crustaceansare first removed and then ground into powder, which is further processed to produce chitosan. Chitosan also occursnaturally in some microorganisms such as fungi and yeast [66].

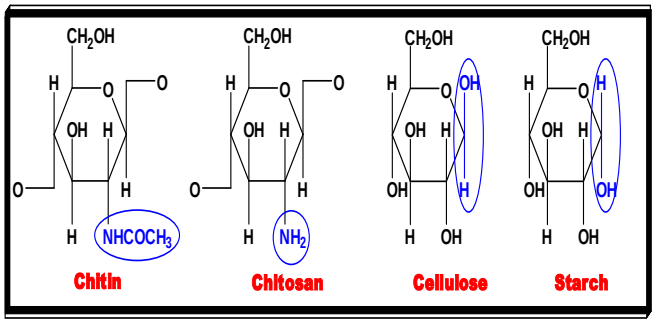
Figure(3):Different sources of chitosan (exoskeleton of crustaceans).

The presence of reactive primary amino groups renders special property that makes CS very useful in pharmaceutical applications. Commercially available CS has an average molecular weight ranging between 3800 and 20,000 Daltons and is 66 to 95% deacetylated. CS has a large number of applications in pharmaceutical dosage form.

Although chitosan is structurally similar to cellulose, it contains, in addition to hydroxyl groups, acetylamine or free amino groups, which display very different properties fromthose of cellulose. Chitosan has attracted attention because of its biological properties and effective uses in the medical field, food industries, and agricultural sector[67]. It shows a variety of biological activities such as phytoalexin elicitor activity, activation of immune response, cholesterol lowering activity, and antihypertension activity. Similarly, mesoporoussilica nanoparticles (NPs) have the ability to efficiently entrap cargo molecules because of their unique characteristic of having a huge pore size. They have already been recognized as a promising drug carrier and have recently become a new area of interest in the field of biomedical applications· For instance, Zhu et al[68]focused on the stimuli-responsive controlled-release systems that responded to tumor cell environmental changes, such as pH, glucose, adenosine-50- triphosphate, glutathione, and H_2O_2. Chitosan's therapeutic properties have also been reported by other researchers, such as inhibition of growth of microorganisms and pain alleviation[69]and promotion of hemostasisand epidermal cell growth. However, some researchers are interested in the potential applications of chitosan for medical and pharmaceutical purposes. Theincreased interest in chitosan, particularly its use in the pharmaceutical field, is attributed to its favorable properties such as biocompatibility, ability to bind some organic compounds, susceptibility to enzymatic hydrolysis, and intrinsic physiological activity combined with nontoxicity and heavy metal ions[70]. These properties are particularly amenable to a wide variety of biomedical applications in drug delivery and targeting, wound healing, and tissue engineering, as well as in the area of nanobiotechnology. Chitosan has attracted attention as a material for drug delivery biomedical applications in the past few years because of its biological and physicochemical properties, leading to the recognition of chitosan as a drug delivery element and a promising material specifically for the delivery of macromolecules. In this regard, chitosan-based delivery systems range from microparticles to NP composites and films. However, there are several drawbacks in the use of chitosan for drug delivery systems. The main drawback is its poor solubility at physiological pH owing to the partial protonation of the amino groups, thereby causing presystemic metabolism of drugs in intestinal and gastric fluids in the presence of proteolytic enzymes. To overcome these inherent drawbacks, various derivatives of chitosan such as carboxylated, different conjugates, thiolated, and acylated chitosan have been used in drug delivery systems. Researchers reported on the goals of using chitosan as an excipient for drug delivery systems[71,72]. Therefore, the main objective of this review is to highlight and investigate the application of chitosan and chitosan-based NP composites in drug delivery systems and to provide some insight for its future potential properties of chitosan.

Preparation and physicochemical properties of chitosan:

Chitosan is recognized as a linear binary heteropolysaccharide composed of b-1,4-linked glucosamine with various degrees of N-acetylation of glucosamine residues. It is prepared from chitin by alkaline N-deacetylationusing concentrated sodium hydroxide (NaOH) solutions at high temperatures for a long period. Another method for the production of chitosan is N-deacetylation using enzymes under relatively mild conditions[73]figure (4).



Figure(4): Structures of Chitin, Chitosan, Cellulose, and Starch respectively.

The commercially available chitosan is mostly derived from chitin of crustaceans by alkaline N-deacetylation because it is easily obtainable. The production of chitosan involves a two-step process. The first step is extraction of chitin [a linear chain consisting of Nacetyl-D-glucosamine (2-acetamido-2-deoxy-b-D-gluconopyranose) joined together by b (1/4) linkage

and removal of calcium carbonate ($CaCO_3$) from crustaceans' shells using dilute hydrochloric acid and deproteination with dilute aqueous NaOH. In the second step, 40-50% aqueous NaOH at 110-115^{0}C is used for deacetylation of chitin for several hours without oxygen. When the degree of deacetylation exceeds50%, then chitosan is produced. The degree of deacetylation and molecular weight are the two fundamental parameters that can affect the properties and functionality of chitosan. These properties include solubility, viscosity, reactivity of proteinaceous material coagulation, and heavy metal ion chelation, and physical properties of films formulated using chitosan such as tensile strength, elasticity, elongation, and moisture absorption[74]. Chitosan is soluble in aqueous acidic solutions, but insoluble in both water and alkaline solutions. The majority of polysaccharides are usually found neutral or negatively charged in an acidic environment. When dissolved, the amino groups (-NH_2) of the glucosamine are protonated to -NH_3, and the cationic polyelectrolyte readily forms electrostatic interactions with other anionic groups. Therefore, the cationic chitosan molecule interacts with negatively charged surfaces that modify its physicochemical characteristics. These modifications of chitosan molecules are the source of its unique functional properties[75].

Specification of Chitosan[76]:

Table 1 shows specification concerning Chitosan

Parameters	Description	Instrument
Appearance (powder or flake)	White or yellow	External shape estimation
Particle size	Less than 30 µm	Optical microscopy
Viscosity (1% solution/ 1% acid)	Less than 5 cps	Intrinsic viscosity (Capillary test)
Density	between 1.35 to 1.40 g/cm3	Densitometer
Molecular weight	50,000 to 2,00,000 Da.	HPLC
pH	6.5 to 7.5	pH meter
Moisture content	More than 10 %	Gravimetric analysis
Ash value	More than 2 %	Gravimetric analysis
Matter insoluble in water	0.5 %	-
Degree of deacetylation	66 % to 99.8 %	(FTIR test)
Heavy metal (Pb)	Less than 10 ppm	
Heavy metal (As)	Less than 10 ppm	
Protein content	Less than 0.3 %	Kjeldal method
Loss on drying	Less than 10 %	
Glass transition temperature	203°C	

Biological Properties:

Chitosan has excellent properties such as hydrophilicity, biocompatibility, biodegradability, antibacterial and adsorption applications, and a very low toxicity[77]. The biocompatibilityof chitosan is generally regarded as the ability of the newly developed material to interact with living cells, tissues, or organs by not being toxic or injurious and not triggering immunological reactions or rejections while functioning appropriately in vitro and in vivo[78].

During the last two decades CS has been used as a safe excipient in drug formulations. Due to its bioadhesive property, it can adhere to hard and soft tissues and has been used in dentistry, orthopedics and ophthalmology and in surgical procedures. It adheres to epithelial tissues and to the mucus coat present on the surface of the tissues. It also has a fungistatic or bacteriostatic, anticancerogen and anticholestermic action. Clinical tests of CS has been carried out in order to promote CS-based biomaterials do not report any inflammatory or allergic reactions following implantation, injection, topical application or ingestion in the human body.

Toxicological properties:

Biodistribution, in vivo and in vitro toxicity using various chitosans of different molecular weights and degrees of deacetylation and derivatives would provide data that could help correlate chitosan's structure and safety profile. Some derivatives increase in toxicity and any residual reactants must be carefully removed. In

laboratory mice, the LD_{50} of chitosan is similar to that of salt or sugar (16 g/kg of body weight)[79].

Drug Release and Release Kinetics from CS based dosage form:

The release of drug from CS based dosage form depends upon the morphology, size, density and extent of cross-linking of the particulate system, physicochemical properties of the drug as well as the polymer characteristics such as either it is hydrophilic or hydrophobic, gel formation ability, swelling capacity, muco-adhesive or bioadhesive properties and also on the presence of other excipient present in the dosage form. In vitro release of drug from the prepared dosage form in the dissolution media also depends upon volume of dissolution medium, pH and polarity, rate of stirring, temperature, sink condition and presence of enzyme. The release of drug from CS particulate systems involves three different mechanisms: (a) erosion, (b) by diffusion and (c) release from the surface of particle. The release of drug mostly follows more than one type of mechanism. In case of release from the surface, adsorbed drug dissolves rapidly and it leads to burst effect when it comes in contact with the release medium. He et al.[80] observed that CS based microspheres prepared by spray drying technique have shown burst release of cimetidine. The burst release of drug can be prevented by use of cross linking agents such as glutaraldehyde and formaldehyde or by washing microparticles with a proper solvent. Al-Helw et al.[81]observed that a high release of the phenobarbitone in initial hours and drug release rate was dependent on the molecular weight of CS and particle size of the microspheres. The microspheres prepared from high molecular weight CS have shown slow release of drug as compared to those prepared from low molecular weight CS. This is due to the fact that high molecular weight CS has lower solubility and formation of the high viscosity gel layer around the drug particles upon contact with the dissolution medium. Microspheres having the size range of 250-500 µm, the release of drug were 75-95% up to 3h but for particles having the size range of 500-1,000 µm, drug release was 56-90% in 5h Ritger and Peppas[82] has given equation for diffusion- controlled matrix system in which the early time release data can be fitted to obtain the diffusion parameters (equation1),

$(M_t/M_\infty) = kt^n$

M_t/M_∞ is the ratio of drug concentration released at time t, k is a constant characteristic of the drug and polymer interaction and n is an empirical parameter characterizing the release mechanisms. Based on the diffusion exponent drug release is classified as Fickian (n=0.5), non-Fickian or anomalous (0.5<n<1), Case II transport (n =1) and super Case II (n>1). Jameela et al.[83]observed a linear relationship between amount of drug released and the square root of time, indicating that the release is diffusion-controlled and obeys Higuchi equation.

Role of Chitosan: a good candidate for ocular drug delivery

The residence time of a topically applied ophthalmic drug refers to the duration of its contact with the ocular surface. This concept is of particular interest in the formulation of topical ocular drug vehicles, where mucoadhesive polymers are frequently used as an approach to prolong drug residence times. When using a mucoadhesive material, the clearance of the drug is controlled by the mucus turnover rate, which is much slower than the tear turnover rate. This prolonged retention of the drug formulation implies, for a drug with good permeability properties, an enhanced ocular drug bioavailability.

Chitosan is in this category of mucoadhesive polymers. The mucoadhesive character of chitosan relates to the attraction between its positively charged amino groups and the negatively charged residues of sialic acid in the mucus along with other forces such as hydrogen bonds[84]. In addition to this special property ,chitosan exhibits other attractive features, which make it a unique candidate for ocular drug deliver.

- It has penetration-enhancing properties, which were initially attributed to the modulation of the tight junction barrier between epithelial cells[85].
- And recently also related to intracellular routes[86].
- More specifically, using Caco-2 cells, that chitosan increases cell permeability by affecting both paracellular and intracellular pathways of epithelial cells in a reversible manner, without affecting cell viability or causing membrane wounds. This permeability-enhancing property has been used to explain the increased corneal transport of specific drugs.
- Chitosan is biodegradable, which enables the safe administration and degradation of topically applied ocular chitosan vehicles. Chitosan biodegradation is mediated by the hydrolytic actions of lysozyme and other enzymes (i.e. human chitinase and Nacetyl-D-glucosaminidases), which produce chito-

- oligomersand monomers[87]. This susceptibility to enzymatic depolymerizationis an exclusive characteristic of chitosan with respect to other polysaccharides.
- Chitosan has excellent ocular tolerance and low toxicity. This has been reported in a rabbit model following topical application of chitosan solutions and using confocal laser scanningophthalmoscopy combined with corneal fluorescein staining.
- Chitosan has favourable rheological behavior. Chitosan solutions have shown pseudoplastic and viscoelastic properties. These are very important characteristics, since the pre-corneal tear film has a pseudoplastic character that should not be disturbed by application of liquid formulations. On the other hand, viscoelastic fluids exhibit high viscosity under low shear rate and low viscosity under high shear rate conditions. This behavior is particularly important in ophthalmic formulations since it facilitates the retention while it permit sthe easy spreading of the formulation due to the blinking of the eyelids[88].

In addition to above mentioned, Chitosan forms colloidal particles and is able to encapsulate bioactive molecules, including proteins, genetic drugs and chemical synthetic agents[89]. This occurs via a variety of mechanisms, such as chemical cross linking, ionic cross-linking and ionic complexation. A wide range of chitosan-based amphiphilic copolymers containing hydrophobic and hydrophilic segments have been developed, because they facilitate the spontaneous self-assembly of diverse functional compartment structures such as micelles, vesicles and gels, with promising prospects in biotechnology and pharmaceutics applications[90]. These nanoparticles can then be administered via ocular, oral, nasal, and intravenous routes[91]. Its high affinity for cell membranes renders chitosan a suitable encapsulating agent for liposome formulations[92].

Moreover, biodegradable nanoparticles are frequently used to improve the therapeutic value of various water soluble/insoluble medicinal drugs and bioactive molecules by improving bioavailability, solubility and retention time. These nanoparticle–drug formulation reduces the patient expenses, and risks of toxicity.

Applications of chitosan in various ocular drug delivery systems are given below:

1- Chitosan-based ocular drug delivery systems:

- **Chitosan solutions**

As previously described in this review, the most acceptable dosage forms for topical ocular drug delivery are the liquid forms. The simplest presentation of chitosan in a liquid formulation consists of a chitosan solution, which is sometimes referred to as a hydrogel. Hydrogels are normally defined as polymers that have the ability to swell in aqueous solvents and undergo a liquid ± gel transition. However, in ophthalmology, there is not a clear distinction between hydrogels and highly viscous solutions.

Chitosan solutions can be prepared in different concentrations and using different types of chitosan (different molecular weight, different salts and different deacetylation degree). Preferably, highly deacetylated chitosan (generally more than 60%) is used since the water solubility decreases with reduction in deacetylation. Chitosan solutions have been well characterized in terms of their pseudoplastic and viscoelastic behaviour. Furthermore, a synergism between rheological behaviour and mucoadhesion has also been described[93]. These studies have shown that the viscosity and mucoadhesive properties can be modulated by adjusting the chitosan molecular weight and concentration. Most of the reports on the topical ocular administration of chitosan solutions refer to concentrations in the range 0.5±5% and molecular weight higher than 70±100 kDa. However, it is possible that other molecular weights and concentrations could be used for this specific application. An alternative way to modulate the viscosity and viscoelastic behaviour of chitosan solutions could be through the incorporation of other hydrophilic polymers that are known to interact with chitosan (e.g. hyaluronic acid)., Some years later,a number of chemical derivatives of chitosan have been produced, among them PEG-chitosan, which is already commercially available. These approaches involving other polymers are valid insofar as the new polymers are acceptable for ocular administration. The specific bioadhesive activity of chitosan towards the ocular surface has been confirmed in an ex-vivo study performed using freshly excised cattle cornea and radiolabelled chitosan[94].

Recently, the capacity of chitosan for increasing pre-corneal drug residence times was clearly shown in a rabbit model, using gamma scintigraphy. For this purpose, chitosan gels containing tobramycin and the commercial drug solution were labeled with ^{99m}Tc-DTPA and instilled onto the cornea of conscious rabbits. The pre-corneal retention time was assessed by

determining the eye-associated radioactivity, using a gamma camera. The results showed a 3-fold increase of the corneal residence time of the chitosan solution as compared with that of the commercial drug solution[95]. In addition, at 10 min post-instillation of the commercial solution, all the radioactivity was concentrated in the lachrymal duct, whereas in the case of chitosan formulations, 25±50% of the radioactivity remained associated with the cornea. The results of pre-corneal drainage were very similar irrespective of the chitosan concentration (0.5±1.5%) or molecular weight (160±1930 kDa). On the basis of these results, the authors suggested that the improvement in retention time using chitosan might be due to a saturable bioadhesive mechanism. Hence, they concluded that the use of allow concentration of low-molecular-weight chitosan would be sufficient to provide a significant enhancement of the residence time.

As indicated above, besides their utility as drug delivery vehicles, chitosan solutions have also been investigated as tear substitutes in the management of dry eye disorders[96]. These studies revealed that chitosan solutions are easier to manipulate and provide a more accurate and reproducible administration, as well as a lower incidence of blurred vision and discomfort than conventional hydrogels used at the ocular level. In addition, the antibacterial activity of chitosan could be useful to prevent, as mentioned above, the frequent secondary infections associated with this disorder. Chitosan has also been studied as an ocular drug delivery vehicle for topically applied vancomycin in rabbit, with promising results[97]. It was found that the drug bioavailability in chitosan-containing solutions was of a similar order to that of commercially available products, identifying the material as a cost- effective alternative for vancomycin ocular drug delivery.

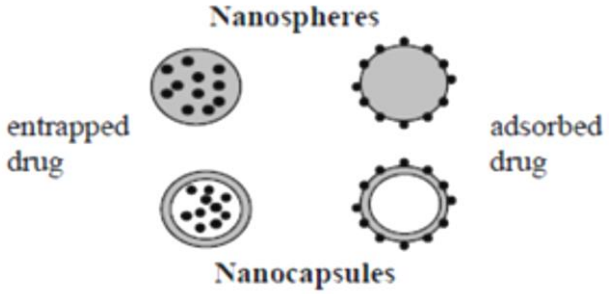
2- Chitosan-based colloidal drug carriers (First Generation**):**

A second generation of ocular colloidal drug carriers is represented by those coated with mucoadhesive polymers. Following the observation of the improvement in transport of drugsassociated with nanocapsules and nanoparticles. Author decided only to explore whether or not a chitosan based (first generation) would further improved their efficacy as ocular drug carriers

Ocular Chitosan Nanoparticles:

- **Properties of Chitosan Nanoparticles:**

Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They consist of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached. Polymers used to form nanoparticles can be both synthetic and natural polymers. There are two types of nanoparticles depending on the preparation process: nanospheres and nanocapsules[98]. Nanospheres have a monolithic-type structure (matrix) in which drugs are dispersed or adsorbed onto their surfaces; and nanocapsules exhibit a membrane-wall structure and drugs are entrapped in the core or adsorbed onto their exterior. The term "nanoparticles" is adopted because it is often very difficult to unambiguously establish whether these particles are of a matrix or a membrane type (figure 7).



Figure(7):Various types of drug loaded nanoparticles

Chitosan has been explored as a material of choice to form nanoparticles (NP) for the last decade[99]. The properties of chitosan have been enhanced by making their nanoparticles. The unique character of NP for their small size and quantum size effect could make CSNP exhibit superior activities They are simple and inexpensive to manufacture and scale-up and have unique size and large surface-to-volume ratio. Nanoparticles are inherently stable structures, in contrast to self-assembled systems. This advantageous stability must however be coupled to a long-term degradation under physiological conditions, in order to prevent undesired body accumulation. Ideally, nanoparticles would deteriorate in products which are naturally excreted, or absorbed by the body. They are mucoadhesive and hydrophilic in nature due to which they provide good protection to encapsulated drug, increase its clearance time and stability in the body. Thus they are applicable to a broad category of drugs; small molecules, proteins and polynucleotides. The benefits of encapsulating active agents in a polymer

matrix include their protection from the surrounding medium or processing conditions and their controlled release.

- **Characteristics of sustained/controlled release[100]:**

Drugs carried by chitosan nanoparticles can be released through degradation and corrosion of chitosan, leading to a clear sustained-release effect. Because of varied degradation rate and time of chitosan of different molecular weight and degree of deacetylation degree, different types of nanoparticles can be used to regulate drug-release rate. Meanwhile, chitosan can also be modified to achieve the sustained/controlled release.

Chitosan–alumino-silicate nanoparticles prepared by Yuan et al.[101]had significant sustained/controlledrelease effects. pH of the environment and chitosan: aluminosilicate ratio also influence drug release.

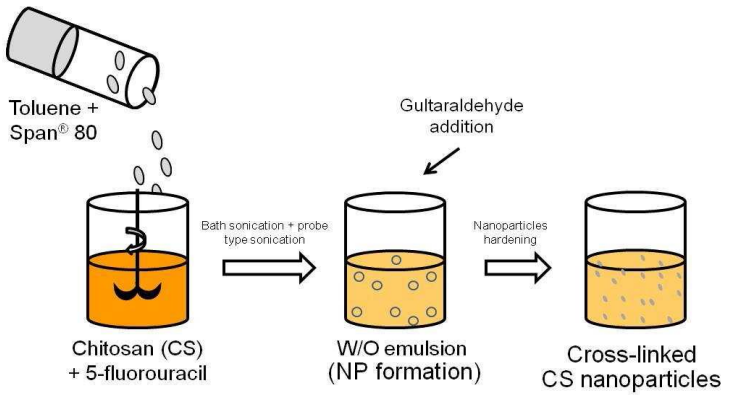
- **Methods used for the preparation of chitosan-based nanoparticles:**

The methods used for the production of chitosan-based nanoparticles and composition of the carriers' matrixare presented in table(2)

Table (2): Methods used for the production of chitosan-based nanoparticles and composition

Production Method	Matrix composition	Reference
Emulsificationand crosslinking	Chitosan, glutaraldehyde	(Songjiang and Lixiang2009)[102]
Emulsion droplet coalescence	Chitosan	(Anto et al. 2011)[103]
Emulsion solvent diffusion	Chitosan	(El-Shabouri 2002)[104]
Reverse micellisation	Chitosan, glutaraldehyde	(Manchanda and Nimesh 2010)[105]
Ionic gelation	Chitosan, tripolyphosphate	(Fan et al. 2012)[106]
Polyelectrolyte complexation	Chitosan, , insulin,	(Kaihara et al.2011)[107]
Modified ionic gelation with radical polymerisation	Chitosan, acrylic acid, methacrylic acid	(Sharma 2006a)[108]
Desolvation	Chitosan	(Atyabi et al. 2009)[109]

The following figures represented the two of above mentioned methods of the preparation of chitosan-based nanoparticles, figures(5,6):



Figure(5): schematic representation of the method of emulsification and cross-linking.

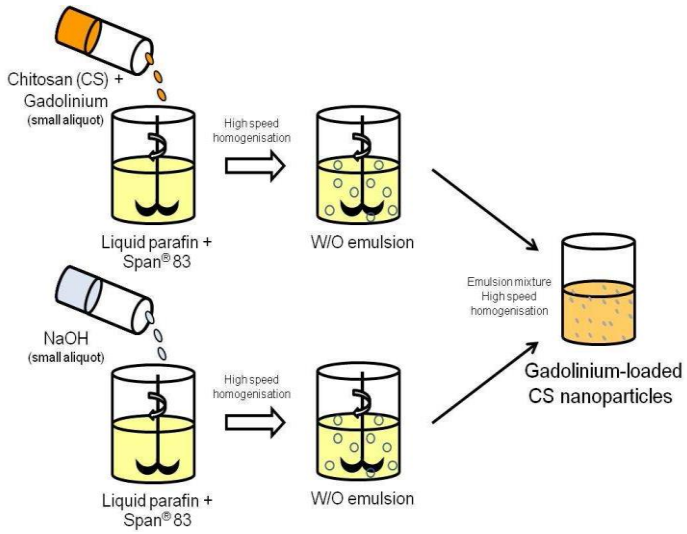


Figure (6):Schematic representation of the method of emulsion droplet coalescence.

- **Applications of chitosan-based colloidal drug carriers:**

Nanoparticles have been found to be potential carriers for ocular delivery following the observation that various types of nanoparticles tend to adhere to the ocular epithelial surface. The resulting prolonged residence time of nanoparticles leads to a much slower elimination rate compared to conventional ophthalmologic formulations, thereby improving drug bioavailability. As a consequence, nanoparticles have been developed for targeted ophthalmic delivery of anti-inflammatory, antiallergic and beta blocker drugs. Felt et al.(1999)found that chitosan solutions prolonged the cornea resident time of antibiotic in rabbits. The same effects were also observedemploying chitosan nanoparticles as demonstrated by De Campos et al. that chitosan NP remained attached tothe rabbits' cornea and conjunctiva for at least 24 hr[110]. Chitosan also shown tobe a low toxic material, ophthalmic formulation based on chitosan exhibited an excellent tolerance after applied chitosan onto the rabbit's corneal surface. Beside employingchitosan NP to improve drug transport via ocular, chitosan nanoparticles are able to interact and remain associated to the ocular mucosa for extended periods of time, thus being promising carriers for enhancing and controlling the release of drugs to the ocular surface.

Cyclosporin A (CyA) was chosen as a model drug. The in vitro release studies, performed under sink conditions, revealed an initial burst release followed by a more gradual drug release during the 24-h period. The in vivo experiments showed that after topical instillation of CyA-loaded CS nanoparticles to rabbits, therapeutic concentrations were achieved in the external ocular tissues (i.e., cornea and conjunctiva) within 48 h while maintaining negligible or undetectable CyA levels in the inner ocular structures (i.e., iris/ciliary body and aqueous humour), blood and plasma. These levels were significantly higher than those obtained following the instillation of CS solution containing CyA and an aqueous CyA suspension. The study indicated that CS nanoparticles could be used as a vehicle to enhance the therapeutic index of the clinically challenging drugs with potential application at the extra ocular level. It was demonstrated the potential of chitosan nanoparticles with cyclosporine A to improve the delivery of drugs to the ocular mucosa. Furthermore, chitosan-based colloidal systems were found to work as transmucosal drug carriers, either facilitating the transport of drugs to the inner eye or their accumulation into the corneal epithelia. The use of chitosan-based colloidal suspensions in vivo showed a significant increase in ocular drug bioavailability[110].

Recently investigated the efficacy of chitosan nanoparticles in prolonging the delivery of drugs to the eye surface. We chose ciclosporin as a model drug that could benefit from a controlled release behaviour at the eye surface due to its potential indication for the treatment of severe dry eye. With this idea in mind, we compared the ocular disposition of three different formulations of 3H-ciclosporin, following topical instillation to conscious rabbits: 3H-ciclosporin-loaded chitosan nanoparticles, a nanosuspension of 3H-ciclosporin in a chitosan solution and an aqueous

nanosuspension of 3H-ciclosporin. Interestingly, chitosan nanoparticles are able to provide a selective and prolonged drug delivery to the ocular mucosa without compromising inner ocular tissues avoiding systemic absorption (De Campos et al 2001). More specifically, following topical instillation of a suspension of 3H-ciclosporin-loaded chitosan nanoparticles, it was possible to achieve significant levels of ciclosporin in cornea and conjunctiva for at least 48 h; these levels were up to 2- to 10-fold higher than those provided by the chitosan solution containing 3H-ciclosporin and the aqueous 3H-ciclosporin suspension. In addition, it was found that the access of ciclosporin to the intraocular structures and blood circulation was restricted by the nanoparticle formulation. These results led us to conclude that chitosan nanoparticles may represent an interesting vehicle for drugs whose target is the ocular mucosa. The specific localization of ciclosporin in the external ocular structures could be related to the mechanism of action and biodistribution of chitosan nanoparticles and also to the inherent properties of this peptide. Indeed, the high accumulation in the cornea must be certainly due to a facilitated interaction of the drug with the corneal epithelium. However, this accumulation may also be favoured by the hydrophobic nature of ciclosporin and, hence its inability to overcome the corneal stroma. The most surprising result was the important accumulation of ciclosporin in conjunctiva together with the lack of systemic absorption. This specific drug retention could be justified by the mechanism of interaction of the particles with the conjunctival epithelium.

El Qidra R. et al., 2008[111] described the development of two different ophthalmic mucoadhesive chitosan based nanocarriers, nanoemulsion and nanoparticles adopting simple and convenient way for indomethacin (IM) ocular delivery. Unlike previously investigated colloidal systems, the interest of these formulations was their ability to interact and remain associated to the ocular mucosa thus prolonging the residence time in the cornea and slow gradual IM release during 24 h was achieved. Furthermore, CS nanoemulsion developed in this study can be proposed as promising carrier to enhance the therapeutic index of clinically challenging IM with potential application at both extra and intra ocular levels.

Zhu X, et al., 2012[112] found that chitosan/thiolated chitosan-sodium alginate NPs (CS/TCS-SA NPs) could deliver greater amounts of drugs into HCE cells and cornea , suggesting they have good potential for ocular drug delivery applications.

More recently, felt et al (1999b) have adapted the ionic gelation technique to produce particles consisting of two polysaccharides with potential interest for ocular drug delivery. Using an experimental design to identify the formulation conditions for obtaining chitosan ± hyaluronic acid nanoparticles. The interest in these new nanoparticles comes from the fact that hyaluronic acid is already being used in ocular drug delivery.

Fluorescent Hyaluronic acid-chitosan NPs prepared by iono-tropic gelation using fluoresceinamine-labeled hyaluronic acid and resuspended in buffer were compatible. Corneal tissue morphology and functionality did not show any changes[113]but, chitosan coated PLA Nano-carrier for topical ocular applications incorporated with 5-fluorouracil showed higher concentrations in aqueous and vitreous humor than free solution and better permeation efficiency for cornea[114].

Chitosan-DNA nanoparticles has the proper nanoparticle size and positive zeta potential charge and can be pharmaceuticals for corneal gene therapy. Hybrid NPs composed of cationized gelatin and the polyanions CS and dextran sulfate (DS) decreases the toxicity to corneal cells and let the NPs safer and more efficient[115].

Furthermore, nanoparticle made of two bioadhesive polysaccharides, hyaluronic acid (HA) and CS, deliver plasmid DNA to the cornea and conjunctiva[116].

Some Technique was Planned to Sustain the Operation Outcomes LDL-mitomycin C-chitosan NPs can be applicable to anti-scarring therapy during excessive conjunctival wound healing[117].

In the present study, theBetaxolol hydrochloride (BH) loading chitosan(CS)-montmorillonite (MMT)/sodium tripolyphosphate (TPP) nanoparticles were prepared successfully by ionic reaction between CS and TPP based on the positively charged amino groups in CS and the negatively charged phosphate groups in TPP. In addition, the hydroxide radical on the surface of montmorillonite interacting with chitosan through hydrogen bond also played an important role during the preparation of nanoparticles. The drug-loaded nanoparticles consisting of MMT, CS and TPP, showed the enhancement of precorneal retention, sustained release,and high drug-loading capacity. The ratio between TPP and CS was critical for the control of particle sizeand encapsulation efficiency of

nanoparticles. No ocular damage in cornea, conjunctiva or iris was observed.Consequently, these nanocarriers represented a promising approach for the circumvention of the present limitationin ocular drug delivery[118].

A similar study, using econazole nitrate as the model drug molecule, was able to demonstrate that spherical chitosan-sulfobutylether-β-cyclodextrin nanoparticles displayed potential as drug carriers in albino rabbits (Mahmoud *et al.* , 2011). Adjustment of process variables, such as the chitosan molecular weight and the concentration of the two ionic agents, allowed for the modification of particle size, polydispersity index, zeta potential, drug content, in vitrorelease and muco-adhesive properties of the resultant nanocomposite[119].

A new method for preparation of thiolated chitosan (TCS) was established, and it achieved a high degree of thiol substitution, of up to 1,411.01±4.02 μmol/g, as determined by Ellman's reagent. In addition, results of the MTT assay (almost 94% cell viability) indicated the safety of TCS toward Human corneal epithelium (HCE) cells. The prepared CS/TCS-sodium alginate nanoparticles (CS/TCS-SA NPs) were characterized by particle size, zeta potential, SEM and mucoadhesion studies. Overall, the results, including smaller size (265.7±7.4 to 471.0±6.4 nm), higher positive charges (49.2±2.3 to 29.5±4.1 mV) and higher mucoadhesion properties, suggest that TCS-SA NPs are more stable and more effective than CS-SA NPs as an ocular drug delivery system[120].

Wanachat Chaiyasan, (2013) has found that CS and dextran sulfate (DS) undergoself-assembly rapidly via ionic interactions under mild conditions resulting in uniformly sized nanoparticles with a positive surface charge and significant entrapment efficacy (EE). The prepared Chitosan–dextran sulfate nanoparticles (CDNs) proved to be stable to lysozyme and showed mucoadhesiveness to the ocular surface. Thus, the CDNs are useful in the treatment of ocular surface diseases, including dry eye and ocular surface infections. In addition, this nanoparticle system can be adoptedfor other drugs to improve residence time, and thereby, intraocular bioavailability for diseases such as glaucoma[121].

Sabitha K.,(2012) has obtained that the formulated moxifloxacin nanoparticles with chitosan as a carrier was found to be a suitable and potential natural carrier in terms of their particle size, zeta potential, drug loadingcapacity, in vitrorelease characteristics and better ocular tolerability. The release profile of moxifloxacin from nanoparticles has shown a sustained release following first order kinetic with non-Fickian anomalous diffusion mechanism. The results demonstrated the effective use of moxifloxacin loaded chitosan nanoparticles as a controlled release preparation for treatment of ocular conjunctivitis infections[122].

Mohamed Ali Attia Shafie (2012) suggested in his study that chitosan alginate nanoparticles would be a promising system for the sustained release deliveryof betamethasone sodium phosphate to the posterior segment of the eye[123].

Kumar Manish (2013), developed and drug loaded nanoparticles of azelastine hydrochloride, an antiallergic drug. Nanoparticles of azelastine hydrochloride (CS–SA nanoparticles) were fabricated using chitosan and sodium alginate as polymers by ionotropic pregelation method. Calcium chloride was also included in the formulation for pregelation of sodium alginate. The potential of CS–SA nanoparticles as drug carriers for ocular delivery was investigated. Cromolyn sodium antiallergic agent used in the treatment of ocular infections, was successfully formulated in the form of CS–SA nanoparticulate system with optimum particle size, zeta potential (>30) maximum entrapment of drug contents[124].

Taskar, P. S.,(2013) was developed chitosan based nanoparticles containing Triamcinolone Acetonide (TA), a corticosteroid administered intravitreally in the treatment of uveitis and ocular inflammation, for topical, non-invasive ocular delivery and to investigate it's in vitro and in vivo characteristics.Chitosan nanoparticles were prepared by cross-linking with sodium tripolyphosphate (TPP) by ionotropic gelation method. Chitosan solutions, 0.25 % w/v and 0.5 % w/v, were prepared in 0.1% acetic acid. TA (0.1% w/v) was dissolved in ethanol and added to the chitosan solution under vigorous stirring. For cross-linking, TPP was added under probe sonication to form chitosan nanoparticles. The ratio of TPP: chitosan was maintained at 1:5. The nanoparticles were characterized in terms of particle size, zeta potential, assay, and entrapment efficiency. Differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) were used to evaluate thermal behavior and possible chemical interactions. Drug release from the chitosan nanoparticles was studied in 20 ml of isotonic phosphate buffered saline; 4% w/v TA suspension was

used as control. The results obtained, in vitro TA release from 0.5% and 0.25% w/v Ch-TA NP was 93% and 98%, respectively, over a period of 2 hours. DSC and FTIR studies did not reveal any significant interaction. The results also have shown that the cross-linked chitosan matrices play an important role in the release of TA from the nanoparticles[125].

Selvaraj. S. et al.(2010) developed and evaluated nanosphere colloidal suspension containing acyclovir as potential ophthalmic drug delivery system. Chitosan nanoparticles had shown an excellent capacity for the association of acyclovir.The acyclovir loaded chitosan nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions (TPP). The mean particle size, morphological characteristics and surface property of the nanoparticlesappear to depend on concentration of acyclovir loaded in chitosan nanoparticles. The release profile of acyclovir from nanoparticles has shown a sustained release following zero orderkinetic with non-Fickian diffusion mechanism. The results demonstrated the effective use of acyclovir loaded chitosan nanoparticles as a controlled release preparation for treatment of ocular viral infections[116].

Andeera D. Moraru, et al. (2014) was evaluated intraocular biodistribution of a fluorescent polymeric nanosystem composed of chitosan and gelatin after intravitreal administration in rat eyes. The nanoparticles obtained using the double crosslinking (covalent and ionic) in double emulsion technique were stable, spherical, highly porous, with relatively high polydispersity submicronic particles. They showed a low tendency to form aggregates and had smooth surfaces in most cases. These properties demonstrate that chitosan gelatin based polymeric nanoparticles may act as viable transporters for intraocular administered drugs. The intravitreal-injected particles are found especially in the lens and retina at 24 hours after administration. Smaller amounts are found in the cornea and sclera at 24 hours after administration. Injected particles are found with greater intensity in the deep retina, vascular and perivascular, especially at 24 hours after administration. At72 hours after the intravitreal injection, although show in glower fluorescence intensity, the particles presence is evident in the lens and retina, but faint inside the corneal and scleral structure. This experimental study demonstrated the ability of fluorescent nanoparticles to penetrate tissue close to the administration site and especially their tendency to migrate deep in the retina at time intervals of 72 hours[117].

Mohammed Mostafa Ibrahim et al.(2013) described the preparation and evaluation of new topical ophthalmic sustain edrelease dosage forms containing celecoxib-loaded CS or Alginates (ALG) NPs prepared by a spontaneous emulsification solvent diffusion method. The optimized NP formulations had desirable particle sizes, zeta potentials and surface morphology. The prepared formulations possessed pH and viscosity values that are compatible with the eye and have uniform drug contents that complied with the USP official requirement. In vitro release data of all ophthalmic formulations showed a sustained release, free of any burst effect; the release profiles follow a Higuchinon-fickian diffusion mechanism. The in vitro cytotoxicity results revealed that all prepared formulations were nontoxic. Statistical analysis of cytotoxicity results showed that there were no statistically significant differences between the formulations and the control preparation that contained 0.1% celecoxib suspension. Although both CS- and ALG-NPs possessed bioadhesive properties, this study suggest that CS-NP preparations will be more efficacious than ALG-NPs due to the positive charge carried by CS. This feature improves its adherence to the negatively charged eye surface, resulting in longer ocular contact time and therefore more sustained effect. Because these formulations are intended for topical use, CS is preferred over ALG due to its penetration enhancing properties. In vivo studies and accelerated stability studies of the ophthalmic formulations stored at three different temperatures (30, 35 and 45°C) for six months are inprogress. The investigation has determined the optimal formulations for sustained delivery of celecoxib to treat conditions of the eye[118].

CONCLUSION

Polymeric materials contribute a significant role in the controlled drug delivery. In particular, biodegradable polymers have been extensively explored for ocular therapeutics in the recent years. This review summarizes mainly the main properties of biodegradable cationic polymer (chitosan) having origin source. In addition, this review emphasizes recent research on different aspects of ocular chitosan based nanodrugs, including the preparation and applications of chitosan nanoparticles.

As a drug delivery system, chitosan nanoparticles have attracted increasing attention because of their good

biocompatibility, degradability, and nontoxicity. Absorption and bioavailability of drug encapsulated into chitosan nanoparticles can be improved, so they can be used in addition to ocular drugs, to deliver protein drugs, gene drugs, and other drugs and can protect them effectively from enzyme degradation in vivo. Chitosan nanoparticles are now being modified for sustained/controlled release and targeting. As the active antitumor components of plant drugs are being constantly discovered and developed, developing targeted chitosan carriers for sustained/controlled release plant drugs is also an area of future development. While great progress has been achieved in the application of chitosan nanoparticles as drug carriers. Many drugs have problems of poor stability, water insolubility, low selectivity, high toxicity, side effects and so on. Clinically useful drug delivery systems need also to deliver a certain amount of a drug that can be therapeutically effective over an extended period of time. Such requirements can be met by the nano scale drug delivery systems. Ocular chitosan NPs are good drug carriers because of their above mentioned properties and can be readily modified. As a new drug delivery system, they have attracted increasing attention for their wide applications. Chitosan nanoparticles are now being modified for sustained/ controlled release and targeting. Finely, although ocular chitosan NPs can be easily modified to carrier, coat and encapsulate hydrophobic drugs, further investigation is required on the biocompatibility of modified chitosan NPs and its derivatives.

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