Chapter 14

Chitosan: A Good Candidate for Sustained Release Ocular Drug Delivery Systems

Lăcrămioara Popa, Mihaela Violeta Ghica,
Cristina Elena Dinu-Pîrvu and Teodora Irimia

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76039

Abstract

This chapter focuses on the eye, one of the most important organs of humans. Current data on pathophysiology of the human eye are presented in direct correlation with a range of therapeutic products, with a well-known and widely used material, namely chitosan. Applications of chitosan biopolymer are described in the development of innovative, modern, therapeutic devices and solutions. Thus, chitosan is a good excipient either for classic drop-type ocular systems, as well as for complex drug systems such as nanostructures (nanoparticles, nanomicelles and nanosuspensions), liposomes, microemulsions, microspheres, in situ hydrogels and inserts or implants. A number of disadvantages for ocular administration of the drugs are thus overcome.

Keywords: chitosan, ocular, delivery systems

1. Introduction

As fascinating as its perfect structure, so difficult to approach due to increased sensitivity and many protective barriers, the human eye continues to be a brainstorming of ideas to formulate and characterize pharmaceutical preparations with optimal action at this level.

The eye can be structured into two large segments: anterior and posterior, the latter representing about two-thirds of the total area. The anterior segment includes the cornea, the conjunctiva, the iris, the lens, the ciliary body and the aqueous humor. Sclera, choroid, retina, vitreous humor and optic nerve are parts of the posterior segment [1].

Following eye drops, the bioavailability of the drug is less than 5% [2] due to factors such as nasolacrimal drainage, lacrimation induction, blink reflexion or corneal barrier [3]. Pharmaceutical formulations given intraocular must be sterile, without pyrogens or endotoxins, isotonic, isohydric and stable. The eye tolerates a pH between 7.5 and 9.5. Alkaline solutions are better supported [4].

Due to the occurrence of diseases such as glaucoma [5], age-related macular degeneration [6], diabetic macular edema [7], diabetic retinopathy [8] or dry eye syndrome [9], which require drug delivery for a prolonged period, it has become necessary to create pharmaceutical formulations that provide sustained release, increased bioavailability with decreased frequency of administration. A significant challenge in achieving this goal is to overcome ocular barriers without causing permanent tissue damage [10].

Introduced on market in 1990, chitosan was the source of numerous studies to harness its potential as pharmaceutical excipient [11]. Obtained by deacetylation of chitin, the second most abundant polysaccharide after cellulose, chitosan consists of D-glucosamine and N-acetyl D-glucosamine linked β-(1-4) [12]. Mucoadhesiveness, biodegradable, biocompatible and non-toxic nature make it a suitable candidate for ocular formulations. Chitosan solutions have pseudoplastic and viscoelectric properties that do not disturb the pre-corneal tear film [13].

New formulations and devices have been obtained to ensure an increased retention time and thus a superior drug delivery system using nanomicelles, nanosuspensions, liposomes, in situ gels, inserts and contact lens [14].

2. Chitosan-based drug delivery systems for ocular administration

2.1. Physiopathology of the eye

The eyeball has a spherical shape and an antero-posterior diameter of about 24 mm. It is structured in to two segments: anterior and posterior (**Figure 1**). The anterior segment of the eye comprises the cornea, conjunctiva, iris and ciliary body, crystalline and aqueous humor [15]. Cornea is transparent, avascular, composed of five layers and provides optimal light transmittance [16]. It continues with sclera through the limbus [17] and the conjunctiva. The conjunctiva is a thin, strongly vascularized, porous [18] membrane where mucus-producing goblet cells are located. The mucin layer interacts with the corneal glycocalyx, facilitating the spreading of the tear film [19]. Aqueous humor provides nutrients needed for the cornea and maintains intraocular pressure at the optimum value [20].

To maintain intraocular pressure at normal values between 12 and 20 mmHg, a proper opening of the anterior chamber angle is required to allow an evacuation of excess through the trabecular meshwork [21]. In the posterior segment of the eye are sclera, choroid, retina, vitreous humor and optic nerve. Choroid has the role of reducing the amount of light that reaches the retina, contributes to thermoregulation through the dissipation of heat and influences the intraocular pressure through the vasculature [22].

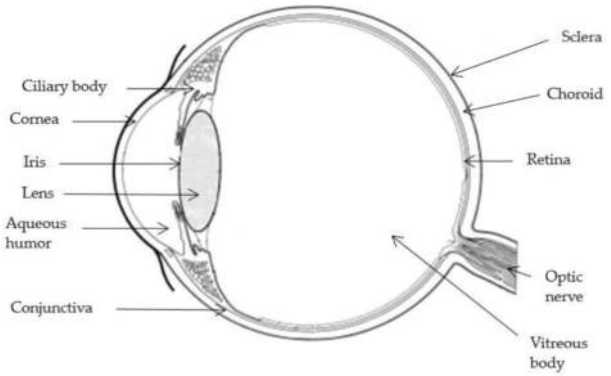

Figure 1. Anatomy of the eye.

The retina is a thin and transparent tissue, made up of 10 layers in which there are two types of receptors: cones and rods. These receptors convert photons into nerve impulse that reaches the brain through the optic nerve [23].

Glaucoma [24–27], conjunctivitis, blepharitis [28], keratitis, dry eye syndrome [29, 30] affect anterior eye segment [31], while posterior segment disorders affecting the vision and even causing complete loss of it: diabetic retinopathy [32], macular degeneration, macular edema and uveitis [33, 34].

Recent studies have made correlations between glaucoma and Alzheimer's disease. Both chronic conditions cause the accumulation of β amyloid associated with inflammatory processes, the appearance of reactive oxygen species and cell apoptosis [35].

The eye is protected by two types of barriers: static and dynamic. Cornea, conjunctiva, ciliary body, aqueous humor and retina are static barriers, while blood flow or lacrimal flow are dynamic barriers. There are situations when their alteration can lead to ocular lesions or hypotonia. The latter consists of penetrating serum proteins into the anterior and posterior rooms with the appearance of edema [36]. Molecules up to 20 kDa can cross the conjunctiva while those up to 5 kDa cornea [37]. In pathological situations, blood retinal barrier alteration causes the permeation of proteins to the retina with the appearance of edema and alteration of vision [38]. In diabetic retinopathy, elevated levels of vascular endothelial growth factor and NO increase the level of reactive oxygen species that generate oxidative stress with neovascularization [39]. The main protector against chemical or microbial aggression is the tear film, a mixture of lacrimal fluid and mucin, an O-glycosylated glycoprotein [40]. It is composed of three different layers [41]. The pH of the tear fluid is about 7.4. It decreases on awakening by the loss of CO_2 resulting from anaerobic metabolism during sleep and increases at contact lens wearers, dry eye syndrome or lacrimal stenosis [42]. Aquaporins play an important role in the transmembranar movements of water through the cornea and conjunctiva in the tear fluid while maintaining the osmolarity of the film [43].

2.2. Chitosan

The benefits of polysaccharides consist of natural abundance, the presence of functional groups available for chemical alterations, and the disadvantages include varied properties depending on the origin, microbial contamination or low microbial resistance [44].

The discovery of chitosan is attributed to Rouget in 1859 when he noticed that he can bring chitin in a soluble form by submitting it to various chemical and thermal treatments [45].

This natural polysaccharide (**Figure 2**) has increased interest because it is non-toxic, biocompatible, biodegradable with various applications in tissue engineering [46–49], food as preservative [50, 51], ruminants' fermentation process [52], in water treatment, medicine and pharmacy as wound dressing [53], implants and medicinal products [54–56]. It is often obtained by deacetylation with an aqueous solution of NaOH from chitin, a polysaccharide from crustaceans' exoskeleton (lobster, crab, squid and shrimp), some fungi and insects [11], insoluble in water but soluble in solutions of dilute acids such as acetic, citric, tartaric and hydrochloric acid at pH < 6.5. It is not soluble in phosphoric or sulfuric acid [57]. This behavior is explained by the protonation of amino groups with the formation of inter-molecular repulsions [11]. It can be dissolved in neutral medium in presence of glycerol-2-phosphate [58].

Biological actions include antimicrobial, antioxidant [59], antiviral [60], antitumoral, antithrombotic and antifungal activity [61]. The positive charge of the molecule binds to the fungal cell membrane, produces an alteration of the K and Ca flux with inhibition of respiration and fermentation [62]. The anti-obesity effect is due to the ability to bind lipids, decreasing their absorption in the digestive tract [63].

Mucoadhesive properties are due to the positive charge that allows interaction with sialic acid from mucin, negatively charged, with the formation of electrostatic bonds [56].

The properties of chitosan are influenced by molecular weight and degree of deacetylation. The biodegradation rate of the polymer is determined by the content in acetyl groups [64]. A degree of deacetylation of 85% or more is preferred due to strong mucoadhesive properties and biocompatibility [65]. In order to obtain oligosaccharides, enzymatic methods are preferred with the use of

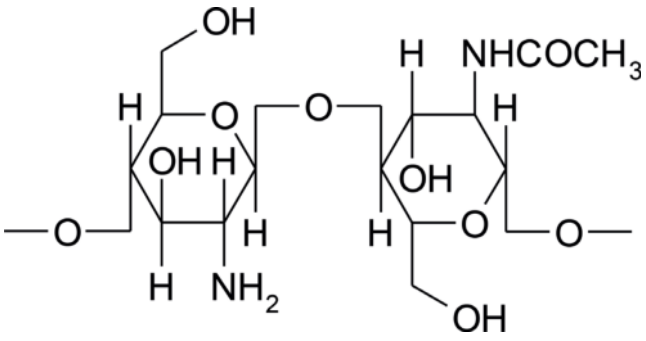

Figure 2. Structure of chitosan.

chitosanases, enzymes with high specificity [66]. Oligosaccharides have anti-inflammatory, antitumoral [67] and antimicrobial action [68].

Low molecular weight chitosan derivatives exhibit water solubility in a wide range of pH, low viscosity and superior biological activities: bactericidal, immunomodulatory, antitumoral, hypolipidemic and hypocholesterolemic [69]. The reactive groups of chitosan are the amino group of C2 and the hydroxyl groups of C3 and C6. Positions C2 and C6 are favorable for substitution. Substitution with carboxymethyl or succinyl groups at this level increases the solubility of the compounds. Due to the presence of a carboxyl group, they can bind calcium, depriving the extracellular matrix of Ca. ions. Thus, they alter tight junctions and its permeability and facilitate paracellular transport through the epithelium. [58]. Chitosan thiolated compounds known as thiomers have strong mucoadhesive properties, increased permeability, antiproteasic activity [70] and inhibit efflux pump [71]. Thiolated derivatives are conjugates with thioglycolic acid or cysteine (**Figure 3**). They exhibit paracellular permeability through the mucosa, forming gels at pH between 5 and 6.8. [72]. Chitosan-N-acetylcysteine has been approved on the market as eye drops under the name Lacrimera, with increased mucoadhesive properties [73].

2.3. Advanced drug delivery technologies

Different strategies have been approached to increase the bioavailability of drug substances at the eye level: increased corneal permeability (prodrugs, permeability enhancers and cyclodextrins), increased viscosity of the vehicle (suspensions, ointments and gels in situ), use of dispersion systems (liposomes, emulsions and nanoparticles), increasing contact time with solid matrix (inserts and contact lenses) [74]. In order to increase eye retention time and reduce the frequency of administration, it is preferred to use natural polymers such as chitosan, gelatin, sodium alginates, sodium hyaluronate, etc. (**Table 1**). At the same time, they are biocompatible, biodegradable and non-toxic [75]. Other advantages of these polysaccharides include natural abundance, nature-friendly materials, relative ease of isolation and low cost [44]. At the same time, they are biocompatible, biodegradable and non-toxic [75]. Other advantages of these polysaccharides include natural abundance, nature-friendly materials, relative ease of isolation and low cost [44].

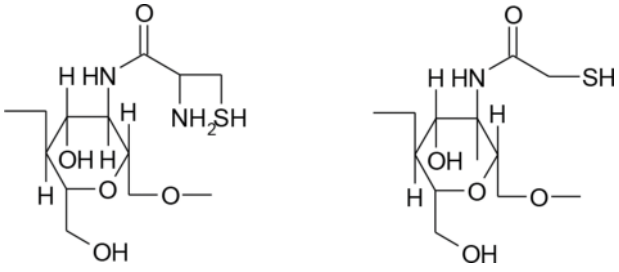

Figure 3. Structures of thiolated chitosans: chitosan-cysteine (left) and chitosan thioglycolic acid.

Polymer	Charge	Solubility	Properties	Ocular dosage forms	References
Chitosan	Positive	Insoluble in water, soluble in solutions of dilute acids such as acetic, citric, tartaric, hydrochloric acid at pH <6.5. It is not soluble in phosphoric or sulfuric acid	Mucoadhesive, biodegradable, biocompatible and non-toxic, pseudoplastic and viscoelastic properties similar to tear film.	In situ gels, nanoparticles, liposomes, micelles microspheres, inserts,	[13, 57, 76]
Sodium hyaluronate	Negative	Soluble in water at room temperature and acidic pH	Biodegradable, viscoelastic properties	In situ gels	[75, 77]
Carrageenan	Negative	Soluble in water, insoluble in organic solvents	Gelling, thickening and stabilizing properties, gelification in presence of Ca^{2+}	In situ gels, microspheres	[58, 75, 78]
Sodium alginate	Negative	Soluble in water, acidic pH. Divalent cations decrease solubility	Gelification in presence of Ca^{2+}, low toxicity, biocompatibility, biodegradability	Ocular mini-tablets, microspheres	[58, 75, 77]
Dextran sulfate	Negative	Soluble in water	Viscosifying, emulsifying, texturizing, stabilizing properties. Excellent biocompatibility and clinical safety	In situ gels	[58, 75, 78]
Colagen	Amphoteric	Soluble in acidic pH	Very compatible with ocular tissues	Ocular films, ocular inserts	[75, 78]
Gelatin	Amphoteric	Soluble in water	Excellent biocompatibility, ease of processing and availability at low cost	Ocular films	[75, 78, 79]
Xanthan gum	Negative	Soluble in water, insoluble in organic solvents	Swelling in basic environment	Viscosity enhancing solutions, gels	[58, 75]

Table 1. Natural polymers used in ocular drug delivery systems to increase eye retention time.

Chitosan increases contact time with cornea, the most commonly used are low molecular weight derivatives [80]. Nanotechnology has been developed to overcome eye barriers and protect active substances [81]. Mucoadhesive nanocarriers increase eye contact time and act as permeability enhancers (**Figure 4**) [82–84].

Thus, innovative formulations have been developed for the anterior segment of the eye, such as preparations based on semifluorinated alkanes applied easy as drops or spray [85], micelles, in situ gels, liposomes, contact lenses [86], inserts [87], dendrimers [88, 89], mini-tablets [90], microspheres [91], nanowafers [92], ocular ring [93] or punctal plug systems [94]. For the posterior segment: micro, nanoparticles, hydrogels, implants and microneedles [95–98].

Characterization of ophthalmic pharmaceutical forms is performed by in vitro and in vivo tests. Determinations include sterility, pH, particle size, viscosity, stability, active substance content and in vitro release. Toxicity studies include the Draize test [99] and the Hen's egg test chorioallantoic membrane (HET-CAM Test) [100]. Particularly, the oxygen permeability is determined for

http://dx.doi.org/10.5772/intechopen.76039

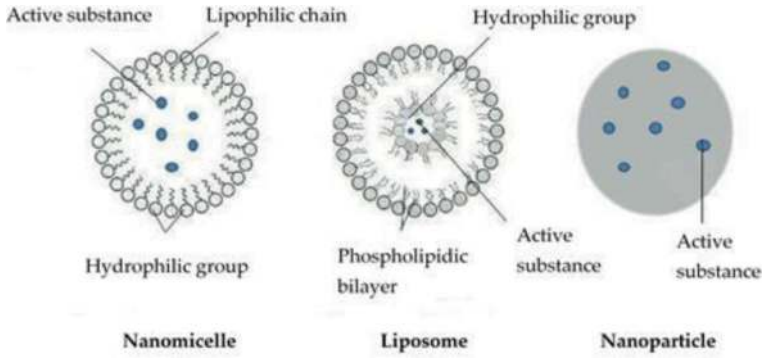

Figure 4. Comparison between different nanostructures.

the lenses, and for the inserts and the contact angle [101]. Measuring the degree of drug release in vitro is vital in the development of a pharmaceutical product, the best known way being with Franz's diffusion cell [102, 103] In a Franz cell, consisting of two compartments separated by an artificial membrane and filled with simulated biological fluid, the formulation to be analyzed is placed. Holding at 37°C, samples are taken at certain time intervals and analyzed to determine the concentration of the substance that crossed the membrane [104].

2.3.1. Nanoparticles

In nanotechnology, the particle size should be between 30 and 200 nm, they should be stable, biocompatible and biodegradable [105]. Chitosan nanoparticles are formed spontaneously by mixing a solution of chitosan with tripolyphosphate (TPP) to form inter and intramolecular bonds. The main mechanism underlying the incorporation of active substances is the occurrence of electrostatic interactions with positively charged chitosan or negative TPP [106].

Basaran et al. have prepared and evaluated chitosan nanoparticles to enhance the ocular permeability of ornidazole for the treatment of bacterial ocular infections. These were prepared by spray-drying method. The nanoparticles were analyzed by morphology, pH, concentration in active substance, in vitro release profile. In 24 h, 98% of the amount of ornidazole was in the simulated biological medium. The authors consider the formulation to be safe and effective for the release of ornidazole at the posterior segment [107].

For the treatment of bacterial endophthalmitis, Silva et al. incorporated daptomycin into chitosan nanoparticles. The preparation was carried out by the ionotropic gelling method, which was subsequently evaluated together with antimicrobial efficiency and stability in the presence of lysozyme and mucin. Using SEM, the particle size was evaluated at about 200 nm. The degree of incorporation varies between 80 and 97%. Total daptomycin release was achieved in 4 h. Incubation with lysozyme did not affect the integrity of nanoparticles [108].

The efficacy of the chitosan-alginate nanoparticles loaded with betamethasone Na phosphate in the treatment of macular edema was studied. With particle size between 16.8 and 692 nm, a rapid initial release was noted, followed by a slow release during 24–72 h [109].

Chitosan nanoparticles were formulated and evaluated by Selvaraj et al. as a potential acyclovir release system at the eye for the treatment of viral diseases. Nanoparticles were prepared by ionic gelling and characterized by SEM, DSC and FTIR. The particle size was between 200 and 495 nm, the encapsulation efficiency was between 56 and 80% and the loading capacity was 10–25%. In vitro release studies demonstrated a sustained release for 24 h, the kinetic release profile following the Higuchi model [110].

The study tracks the potential of montmorillonite in the preparation of prolonged ophthalmic nanoparticles. The nanoparticles were prepared by ionic gelling of chitosan with sodium tripolyphosphate. With a spherical shape between 358 and 585 nm and an incorporation efficiency of between 12.27 and 50.92%, nanoparticles release betaxolol within 10 h, being effective in the treatment of glaucoma [111].

The sustained release of celecoxib from the nanoparticles of chitosan and alginate was proposed by Ibrahim et al. Various blends of polymers were prepared in varying proportions in order to obtain the optimal formulation with the smallest particle size and the highest potential zeta.

Nanoparticles were included in collyria, in situ gels and preformed gel. With TEM, spherical particles with an incorporation efficiency of over 75% have been shown. The release of active substance followed the Higuchi model, and the formulations proved to be non-toxic according to in vivo studies [112].

2.3.2. Nanomicelles

Nanomicelles, amphiphilic molecules that have the ability to form in an aqueous medium organized supramolecular structures, contribute to the solubilization of hydrophobic active substances.

A positive-load nanomicelle increases the retention time and the permeability due to interactions with the negatively charged eye surface. Changing its surface by the addition of a cationic polymer such as chitosan increases contact time to the eye [113].

Another study has proposed the formulation of pluronic/chitosan nanoparticles whose surface has been modified by adding chitosan in order to increase the ocular bioavailability of metipranolol. Nanomicelles were analyzed by diameters, morphology, turbidity, stability and in vitro release. The drug nanoparticle size ranged from 123 to 232 nm with a zeta potential between 6.1 and 9.2 mV. According to the turbidity test, the micelles were stable, preventing the vision from collapsing. The release was 88% in 6 h [114].

A study designed to evaluate rapamycin ocular release from octanoyl-g-chitosan-g-PEG nanomaterials was initiated by Somavarapu et al. Micelle size was determined using dynamic light scattering (DLS), surface morphology with transmission electron microscopy (TEM) and thermal properties with differential scanning calorimetry (DSC). The concentration in the active substance was determined by the HPLC method. Following the study, nanomicelles with a size of 52 nm were obtained and positively charged. The formulation remained stable for 3 days. On visual analysis the preparation is clear with a dispersion index of 0.25. Tissue retention was 24 h [115].

2.3.3. Nanosuspensions

Shi et al. have formulated a chitosan and methoxy polyethylene glycol-poly (β-caprolactone) nanosuspension for the ophthalmic delivery of diclofenac. Nanosuspension was characterized by FTIR, X-ray diffraction and DSC. Nanosuspension was stable at 4 and 25°C for 20 days. Prolonged release of diclofenac was achieved for 8 h without irritation [116].

A nanosuspension of chitosan, sodium alginate and tripolyphosphate was developed as an efficient delivery system of lomefloxacin. Nanosuspension was evaluated for particle size, zeta potential, incorporation efficiency and permeability through the bovine cornea. The incorporation efficiency of the active substance was 70.63%, particle size 176 ± 0.28 nm, zeta potential 13.65 mV. Nanosuspension releases lomefloxacin for more than 8 h and a three-fold increase in bovine corneal permeability to solutions is noted. Also, administration of lomefloxacin in the form of nanosuspension provides the advantage of a prolonged action, protects against enzyme metabolism and increases corneal permeability. Chitosan possesses antimicrobial activity, potentiating the effect of the antibiotic [117].

A chitosan-based nanosuspension with the active substance itraconazole is prepared by co-precipitation. It has been noticed that co-precipitation of itraconazole from the chitosan- lysine system in the presence of poloxamer 100 as a stabilizer causes a nanosuspension with the smallest size, increases drug solubility 12-fold and a very fast in vitro release. Comparative assessment with a commercial suspension determines a significantly increased permeability on the goat's cornea in the first case [118].

2.3.4. Liposomes

Introduced as drug carriers in 1968 [114], liposomes are membrane vesicles composed of one or more phospholipidic or cholesterol layers designed to transport drug substances incorporated either into the core or into one of the layers [36]. They are biodegradable and biocompatible, increasing the permeability of the drug with increasing retention time. These can be administered at both the anterior and posterior segment.

Chitosan-coated liposomes, called chitosomes, increase ocular retention with decreased metabolism of drug substances. Coating liposomes with quaternary ammonium chitosan derivatives such as N-trimethylchitosan reduces particle aggregation due to steric stability and increases mucoadhesiveness [119].

Liposomes with an incorporation efficiency of more than 90% bromfenac were prepared for targeting the retina. Changing liposome surface with chitosan improves mucoadhesive properties. The optimal concentration of chitosan that prevents liposome aggregation was determined at 0.15% [120].

A potential carrier for ocular drug release were low molecular weight chitosan-based liposomes formulated by Li et al. Liposomal morphology was examined with TEM, and cytotoxicity was assessed in rabbit conjunctival cells. By incorporating cyclosporin A, a delayed release profile was revealed as compared to un-coated liposomes. In vivo studies showed that the concentration of cyclosporin in different ocular tissues increased over 24 h [121].

The objective of the study initiated by Ustundag-Okur et al. has been exploiting the potential of nanostructured lipid carriers with chitosan for ocular application of ofloxacin. Particle characterization involved determining the size, potential zeta, viscosity, incorporation efficiency, active substance load or sterility. According to the authors, the system has a 48-h corneal retention time and a substance incorporation efficiency of over 97%. Chitosan improves transcorneal permeability [122].

2.3.5. Microemulsions

The use of microemulsions as drug delivery systems offers advantages such as thermodynamic stability, increased eye retention, improved absorption, incorporation of substances in any of the two phases [123].

Bhosale et al. have formulated several chitosan-based microemulsions as a potential voriconazole release system at the eye level. The formulations were evaluated for thermodynamic stability, physico-chemical parameters, in vitro and in vivo release studies. All the formulations have a particle size of less than 250 nm, potentially zeta positive. In vitro delivery tests have shown that the formulations have a sustained release of over 12 h compared to market formulations. Following in vivo studies in rabbits, it was concluded that the formulations showed an active substance concentration of more than 47% in aqueous humor at 4 h after administration compared to the product Vozole with a voriconazole concentration of approximately 20% [124].

The evaluation of the tear retention of a chitosan-based emulsion containing indomethacin was carried out by Yamaguchi et al. This was compared to a non-chitosan emulsion after instillation in rabbits. The chitosan emulsion has an average concentration of 3.6 and 3.8 higher than that without chitosan at 0.5 and 0.75 h after instillation. The average residence time and half-life for the chitosan emulsion were 1.5 times and 1.8 times higher than the comparative emulsion. It has been appreciated that the chitosan emulsion has a prolonged lacrimal retention time and a wide distribution on the ocular surface due to the mucoadhesive properties of chitosan [125].

2.3.6. Microspheres

Chitosan microspheres determine a controlled release of drug substances and increase the bioavailability of drugs, improving the absorption of hydrophilic substances at epithelial level. They facilitate the transport of substances to the eye or accumulation at the corneal or conjunctival level [126].

Chitosan-based microspheres loaded with ganciclovir were prepared by Kapanigowda et al. Characterization of the formulation was achieved by in vitro release studies, release kinetics and stability of microspheres. The degree of eye irritation, pharmacokinetic parameters and histopathology were evaluated on Wistar rats. In vitro release studies showed an initial burst in the first few minutes, the diffusion following Fick's law. Stability studies were favorable and it was determined that in 75 h, three administrations of this formulation were needed compared to six administrations of ganciclovir as a solution [127].

A study initiated by Rajawat et al. has proposed to develop chitosan and chitosan-N-acetyl cysteine-based microspheres as possible ocular delivery system for acyclovir. The formulations were prepared using emulsification crosslinking process, the microspheres having an active substance incorporation efficiency of 97.86 ± 2.06% for the chitosan microspheres and 76.99 ± 1.14% for the thiolate derivatives. In vitro release studies showed an initial burst followed by a sustained release of acyclovir for 12 h, and in vivo studies did not indicate signs of ocular toxicity [128].

2.3.7. Hydrogels in situ

In situ gels have shown interest since the 1970s.The first gel was synthesized by Kopecek in 1971. It still possesses the "smart" name because they respond to the stimulus by a change in physical or chemical behavior.

Hydrogels are defined as three-dimensional structures that absorb water in large quantities without dissolving into it. Water can not be removed either under pressure [58]. For example, administration of timolol in the form of drops requires two administrations per day, and only one application per day as a gel [129].

Chitosan dissolved in acidic solution and neutralized with β-glycerophosphate undergoes a sol-gel transformation at body temperature, favoring the transfer of protons from chitosan to the weak base.

Because of the amino-positive groups, it is able to interact spontaneously with anionic polymers, forming polyelectrolyte complexes (PECs) with an increased tendency to form hydrogels: chitosan-chondroitin sulfate, chitosan dextran sulfate (**Figure 5**, chitosan alginate [130]. A gel based on chitosan and dextran sulfate was proposed for the ciprofloxacin release study. It has been chemically characterized, morphologically, in terms of stability and concentration in the active substance. Among the analytical techniques used are FTIR, SEM and DSC. Ciprofloxacin release in simulated lacrimal fluid was determined using a UV-Vis spectrometer. The eye tolerance test was evaluated using HET-CAM (Hen's egg test chorioallantoic membrane). The result of the study was a non-irritating product that provides ciprofloxacin release for 21 h in the treatment of susceptible germs infections [131].

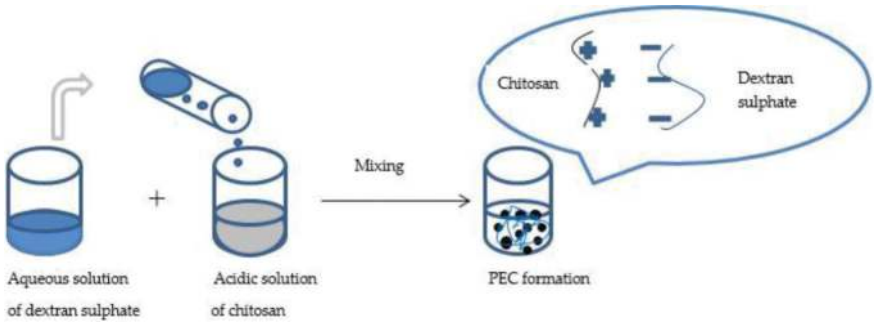

Figure 5. Steps in formation of chitosan-dextran sulfate gel, illustrating the technique described by Jain et al. [131].

The main advantage of this type of gels is the sustained release of the active substance and the absence of blurred vision. Due to the increased contact time with the eye surface, the bioavailability of the active substance is increased, the frequency of administration is reduced [132].

A gel composed of 15% pluronic and 0.1% chitosan with a ciprofloxacin's release efficiency of 46.61 ± 0.41% and a time release of 1.94 ± 0.27 h was developed by Varshosaz et al. Ciprofloxacin release was determined by the dissolution method in artificial tear solution up to 8 h, and the samples were analyzed spectrophotometrically at 272.4 nm. Rheologic behavior and phase transition temperature (PCT) were determined using a Cup and Bob viscometer. The formulation was kept liquid at pH 4 and 25°C and gel transformed to pH 7.4 and 37°C [133].

From several formulations analyzed, Gupta et Vyas proposed a mixture of 0.4% Carbopol and 0.5% chitosan as an optimal ocular drug release system for timolol maleate. It is in a liquid state at room temperature and pH 6 and is a gel under the action of tear fluid at pH 7.4. The formulations were analyzed: pH, viscosity, swelling capacity and concentration in active substance. According to the studies, substance delivery followed Fick's law for 24 h [134].

Zaki et al. attempted to incorporate ketorolac tromethamine into various hydrogels for ophthalmic administration. As polymers, chitosan and Carbopol 940 were used in different concentrations. The visual aspect, pH, viscosity, in vitro delivery behavior and stability were analyzed. The best formulation according to the authors would be the one with 0.5% chitosan in composition [135].

A gel based on chitosan and dextran sulfate was proposed for the ciprofloxacin release study. It has been chemically characterized, morphologically, in terms of stability and concentration in the active substance. Among the analytical techniques used are FTIR, SEM and DSC. Ciprofloxacin release in simulated lacrimal fluid was determined using a UV-Vis spectrometer. The eye tolerance test was evaluated using HET-CAM (Hen's egg test chorioallantoic membrane). The result of the study was a non-irritating product that provides ciprofloxacin release for 21 h in the treatment of susceptible germs infections [136].

The aim of a study initiated by Gilhotra et al. is to evaluate the alginate-chitosan eye film with atenolol in the treatment of glaucoma. The study showed that the addition of Ca gluconate leads to an increased release of atenolol from the chitosan-alginate matrix without the desired sustained effect [130].

Another study proposes a corneal membrane composed of chitosan and collagen. The membrane was prepared by dissolving chitosan in collagen in varying proportions, followed by the addition of 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide as a crosslinker. The membrane was characterized in terms of mechanical properties, contact angle and optical transmittance. In vitro cell culture studies have shown that collagen does not influence cell morphology, viability with good compatibility [137].

Fabiano et al. formulated a chitosan and β-glycerophosphate gel for incorporation of transcorneal 5-fluorouracil nanoparticles. The sol-gel transition takes place in the range of 30–35°C. The concentration in active substance is kept constant for 7 h after administration. The system is a potential candidate for optimal 5-fluorouracil release at eye level [138].

2.3.8. Inserts and implants

Intravitreal injections are the most common method of administering drugs to the posterior segment of the eye. They can be indicated in conditions such as age-related macular degeneration (AMD) with monoclonal antibodies such as bevacizumab (Avastin) or ranibizumab (Lucentis).

An alternative to injections is ophthalmic implants such as Vitrasert (ganciclovir), Retisert (fluocinolone acetonide), Iluvien (fluocinolone acetonide) and Ozurdex (dexamethasone) [139]. Ozurdex is bioerodible [140].

Ophthalmic inserts are solid, semi-solid, sterile, thin, multilayer, impregnated with active substance and placed on the conjunctival sac. Following studies, they have demonstrated increased retention time, sustained release for a longer period of time, dosage accuracy, reduced frequency of administration and lack of preservatives with irritant potential. They can be classified as solubles (with natural or synthetic polymers, insolubles (Ocusert—diffusion mechanism of release; or soft contact lenses—osmosis mechanism) and bioerodibles (Lacrisert) 6 [141].

Chitosan-based ocular inserts have been designed as an alternative to the release of brimonidine tartrate in the treatment of glaucoma. Characterization of inserts was performed from an analytical point of view using FTIR, SEM and DSC. Swelling capacity, active substrate release profile, in vitro bioavailability on Muller cells were also studied. The results of the study were that brimonidine tartrate was physically dispersed between the polymer chains. The inserts release the active substance for 30 days without adverse effects. They also have the advantage of being free of preservatives [142].

Foureaux et al. studied the effects of some antiglaucoma inserts from chitosan. The inserts having diminazene aceturate as active substance were prepared by casting technique and analyzed for swallow capacity, analytically for FTIR, DSC and SEM. Quantification of the active substance from the inserts was performed with the UV-Vis spectrometer and in vitro release studies using a Franz cell. The authors concluded that inserts reduce intraocular pressure by up to 4 weeks [143].

Upadhyaya et al. prepared chitosan-based inserts by casting method for levofloxacin release at the eye level. It has been observed that PVP addition increases levofloxacin release rate. Based on in vitro delivery studies, it was concluded that ocular inserts are suitable for the release of the active substance over 24 h and are useful in the treatment of bacterial infections [144].

The purpose of the study initiated by Franca et al. is to evaluate the effectiveness of some chitosan-based inserts with bimatoprost. The sustained release of the active substance is performed according to in vitro studies at 8 h, which recommends it as a potential alternative in the treatment of glaucoma [145].

2.3.9. Contact lenses

Theoretically, ocular administration of active substances through contact lenses is 35 times more effective than eye drops.

Soft contact lenses are generally made of hydrogels due to their biocompatibility and transparency.

Incorporation of the active substances is accomplished by wetting the lenses with a drug solution, inclusion in a polymeric mixture or in a colloidal structure such as nanoemulsion, nanosuspension, liposomes dispersed in the lens, ligand grafting on the hydrophilic matrix with the formation of inclusion complexes with the drug [146]. If the drug's affinity for the lens is too high, the formulation is stable, but the release is difficult. If the drug is weakly retained by the lens, the release is rapid, followed by a steep decline [147].

Hydration is required when using contact lenses, allowing oxygen to penetrate the cornea. Since the lack of hydration results in dry eye syndrome [148], it is recommended to use contact lenses in association with eye drops [149].

Several advantages are attributed to the use of hydrogel contact lenses: good light transmission, chemical stability and high mechanical properties, increased permeability for oxygen [150].

Behl et al. proposed to increase eye bioavailability of dexamethasone by incorporating it into chitosan nanoparticles which were subsequently imprinted in pHEMA hydrogel contact lenses. Particle size was analyzed by SEM, interactions between dexamethasone and nanoparticles by FTIR. They also studied in vitro release studies. Obtaining an average transmittance of 95–98% demonstrates lens clarity, and dexamethasone release was 55.75% in 22 days. According to the study, the bioavailability of dexamethasone was 72% compared to eye drops within the first 10 days. The conclusions of the study were that the application of contact lenses with chitosan nanoparticles in which dexamethasone was incorporated, leads to therapeutically positive responses [151].

The association of chitosan and gelatin has been shown to be beneficial in the preparation of contact lenses according to Xin-Yuan et al. The film was characterized by permeability, transmittance, water absorption and mechanical properties. The study demonstrated that the film is biocompatible, transparent, permeable and gelatin association has increased water absorption and oxygen permeability [152].

Wearing contact lenses can create certain problems, so Hu et al. have proposed the assembly of a chitosan/hyaluronic acid multilayer on the surface of the lens in order to improve the surface properties such as wettability or deposition of proteins. The chitosan/hyaluronic acid multilayer was loaded with norfloxacin and timolol, respectively. It was observed that the multilayer steadily releases norfloxacin in 1 h, and timolol in 30 min. The purpose of this study is to increase the hydrophilic character of the lenses, increase the water retention and reduce the deposition of the proteins [153].

2.3.10. Mini-tablets

Mini-tablets are devices with a diameter of approximately 2–4 mm inserted into the conjunctival sac. They can gel in the presence of lacrimal fluid or the matrix can dissolve, releasing the active substance [154].

Among the advantages of mini-tablets are easy administration, increased compliance, sustained release, lack of irritation and lack of dilution of drug substance [155].

EL-Gawad et al. prepared ocular mini-tablets based on various polymeric matrices including chitosan for the controlled release of piroxicam. The friability studies showed a 2.36% weight loss in the chitosan mini-tablets, which means they can resist the stresses that occur when administered without producing a foreign body sensation. They also have the ability to quickly disintegrate when administered [156].

Refai and Tag aimed to formulate and evaluate some aciclovir eye mini-tablets to treat keratitis. The spongy nature of the mini-tablets provides fast hydration and gelling at the eye level, reducing foreign body sensation. Several mini-tablets with different polymers including chitosan have been evaluated. Rheological studies have shown pseudoplastic behavior. Optimal release of acyclovir was in the case of chitosan mini-tablets. The chitosan mini-tablets were chosen for the significant sustained release of acyclovir and bioadhesive properties, and the corneal permeability is superior to the Zovirax ointment [157].

Verestiuc et al. were prepared acrylic-functionalized chitosan hydrogels with N-isopropyl acrylamide or 2-hydroxyethyl methacrylate monomers, then pressed to obtain mini-tablets. These have been evaluated for the controlled release capacity of some drugs at the ophthalmic level. By comparison, interpolymeric complexes and pure chitosan were analyzed. The effects of the structure and composition of the network on the properties of swelling, adherence and release of active substances such as chloramphenicol, atropine, pilocarpine or norfloxacin were studied. In vivo studies in rabbits which received pilocarpine indicated that mini-tablets based on chitosan and 2-hydroxyethylmethacrylate are optimal carriers for the delivery of the therapeutic agent [158].

Another study aims to develop and study mini-tablets of sodium alginate, calcium gluconate and chitosan for the purpose of ocular delivery of gatifloxacin. In vivo tests and irritation studies were performed on rabbits. The release was 95–99% on 6–24 h according to the authors. It has been observed that this is enhanced by the increased addition of calcium gluconate. Also, the mini-tablets have been found to be non-irritating and the chitosan and alginate mini-tablets have good antimicrobial properties [159].

3. Conclusions

The human eye is a small, sensitive and complex organ that represents a continuous challenge in pharmaceutical research. The reduced bioavailability (below 5%) of drug substances as eye drops due to factors such as nasolacrimal drainage, blinking reflexes or ocular barriers has made it necessary to develop new ways of administration. Due to its properties, chitosan is considered a good candidate as an excipient in various pharmaceutical formulations for ocular administration. It is biocompatible, biodegradable and non-toxic. It has mucoadhesive properties by interacting with sialic acid residues from the mucin structure and pseudoplastic and viscolectric properties similar to lacrimal fluid. Thiolated derivatives, called thiomers, have enhanced mucoadhesive properties and improve the permeability of active substances through ocular barriers.

The use of chitosan in ophthalmic delivery systems such as nanoparticles, nanomicelles, nanosuspensions, liposomes, microemulsions, microspheres, in situ gels, inserts, contact lenses or mini-tablets increases the retention time of the active substance at the eye level with enhancing its bioavailability. Thus, it will decrease the frequency of administration and will increase patient's compliance with improving his quality of life. These chitosan-based systems do not cause irreversible alterations in ocular barriers, do not damage the tissues, or interfere with tear fluid.

Author details

Lăcrămioara Popa, Mihaela Violeta Ghica*, Cristina Elena Dinu-Pîrvu and Teodora Irimia

*Address all correspondence to: mihaelaghica@yahoo.com

Faculty of Pharmacy, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania

References

[1] Cholkar K, Dasari SR, Pal D, Mitra AK. Eye: Anatomy, physiology and barriers to drug delivery. In: Mitra AK, editor. Ocular transporters and receptors: Their Role in Drug Delivery. Cambridge: Woodhead Publishing Limited; 2013. pp. 1-36. DOI: 10.1533/9781908818317.1

[2] Rupenthal ID. Ocular drug delivery technologies: Exciting times ahead. Opthalmic Drug Delivery. Jan 2015;**54**:7-11

[3] Suresh C, Abhishek S. pH sensitive in situ ocular gel: A review. Journal of Pharmaceutical Science and Bioscientific Research. 2016;**6**(5):684-694

[4] Cojocaru IC. Forme farmaceutice oftalmice. In: Popovici I, Lupuleasa D, editors. Tehnologie farmaceutica. 4th ed. Iasi: Polirom; 2017. pp. 664-717

[5] Agarwal R, Gupta SK, Agarwal P, Saxena R, Agrawal SS. Current concepts in the pathophysiology of glaucoma. Indian Journal of Ophthalmology. 2009;**57**:257-266. DOI: 10.4103/0301-4738.53049

[6] van Lookeren Campagne M, LeCouter J, Yaspan BL, Ye W. Mechanisms of age-related macular degeneration and therapeutic opportunities. The Journal of Pathology. 2014;**232**: 151-164. DOI: 10.1002/path.4266

[7] Klaasen I, Van Noorden CJF, Schlingemann RO. Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions. Progress in Retinal and Eye Research. 2013;**34**:19-48. DOI: 10.1016/j.preteyeres.2013.02.001

[8] Tarr JM, Kaul K, Chopra M, Kohner EM, Chibber R. Pathophysiology of diabetic retinopathy. ISRN Ophtalmology. 2013. Article ID 343560. 13 p. DOI: 10.1155/2013343560

[9] Hessen M. Dry eye: Master the science beneath the surface. Review of Optometry. 2017; **154**(4):56-62

[10] Kumar V, Rajput R, Singh S. The use of in situ hydrogel in ocular drug delivery. International Journal of Pharma Professional's Research. Jan 2016;**7**(1):1319-1325

[11] Raafat D, Sahl HG. Chitosan and its antimicrobial potential – A critical literature survey. Microbial Biotechnology. 2009;**2**(2):186-201. DOI: 10.1111/j.1751-7915.2008.00080.x

[12] Cheung RCF, Bun Ng T, Wong JH, Chan WY. Chitosan: An update on potential biomedical and pharmaceutical applications. Marine Drugs. 2015;**13**:5156-5186. DOI: 10.3390/md13085156

[13] Alonso MJ, Sanchez A. The potential of chitosan in ocular drug delivery. Journal of Pharmacy and Pharmacology. 2003;**55**:1451-1463. DOI: 10.1211/0022357022476

[14] Patel A, Cholkar K, Agrahari V, Mitra AK. Ocular drug delivery sistems: An overview. World Journal of Pharmacology. 2013;**2**(2):47-64. DOI: 10.5497/wjp.v2.i2.47

[15] Barar J, Aghanejad A, Fathi M, Omidi Y. Advanced drug delivery and targeting technologies for the ocular diseases. BioImpacts: BI. 2016;**6**(1):49-67. DOI: 10.15171/bi.2016.07

[16] Remington LA. Clinical Anatomy and Physiology of the Visual System, 3rd ed. St. Louis: Elsevier/Butterworth-Heinemann; 2012. 297 p

[17] Agrahari V, Mandal A, Vivek A, Trinh HM, Joseph M, Ray A, Hadji H, Mitra R, Pal D, Mitra AK. A comprehensive insight in ocular pharmacokinetics. Drug Delivery and Translational Research. 2016;**6**:735-754. DOI: 10.1007/s13346-016-0339-2

[18] Azari AA, Barney NP. Conjunctivitis: A systematic review of diagnosis and treatment. Journal of the American Medical Association. 2013;**310**(16):1721-1729

[19] DelMonte DW, Kim T. Anatomy and physiology of the cornea. Journal of Cataract and Refractive Surgery. 2011;**37**:588-598. DOI: 10.1016/j.jcrs.2010.12.037

[20] Goel M, Picciani RG, Lee RK, Bhattacharya SK. Aqueous humor dynamics: A review. Open Ophthalmology Journal. 2010;**4**:52-59. DOI: 10.2174/1874364101004010052

[21] Ansari MW, Nadeem A. The eyeball: Some basic concepts. Atlas of Ocular Anatomy. 2016:11-27. DOI: 10.1007/978-3-319-42781-2_2

[22] Addo E, Bumiro OA, Siwale R. Anatomy of the eye and common diseases affecting the eye. In: Addo RT, editor. Ocular Drug Delivery: Advances, Challenges and Applications. 1st ed. Cham: Springer International Publishing; 2016. pp. 11-24. DOI: 31 10.1007/978-3-319-47691-9_2

[23] Irsch K, Guyton DL. Eye features and anatomy. In: Li SZ, Jain AK, editors. Encyclopedia of Biometrics. Boston, MA: Springer; 2015. pp. 11-16. DOI: https://doi.org/10.1007/978-1-4899-7488-4_9172

[24] Ayyagari R. Glaucoma and vitamin D. In: Weinreb RN, editor. International Glaucoma Review. The Journal of the World Glaucoma Association. Amsterdam: Kugler Publications. The Netherlands. 2017;**18-2**:52

[25] Yee S. Glaucoma therapy: Finding the right combination. Review of Optometry. 2017; **154**(4):46-52

[26] Brennan S. Lowering IOP: Will first-line options change? Review of Ophtalmology. 2017; **24**(6):30-34

[27] Kresch S. Reinventing glaucoma therapy. Review of Optometry. 2017;**154**(2):40-45

[28] Jordan L. Blepharitis: Know what to look for. Review of Ophtalmology. 2017;**24**(10):36-41

[29] Kwan J. A comprehensive look at dry eye therapy. Review of Optometry. 2017;**154**(5):91-97

[30] Hauser W. Dry eye: A young person's disease? Review of Optometry. 2017;**154**(2):60-64

[31] Ahmed TA, Aljaeid BM. Preparation, characterization and potential application of chitosan, chitosan derivatives and chitosan metal nanoparticles in pharmaceutical drug delivery. Drug Design, Development and Therapy. Jan 2016;**10**:483-507. DOI: 10.2147/DDDT.S99651

[32] Greven MA, Winston-Salem Do DV, Alto P. Using VEGF inhibitors for diabetic retinopathy. Review of Ophtalmology. 2017;**24**(7):61-64

[33] Gower NJD, Barry RJ, Edmunds MR, Titcomb LC, Denniston AK. Drug discovery in ophtalmology: Past success, present challenges and future opportunities. BMC Ophtalmology. 2016;**16**:11

[34] Berthke W. The latest treatment approaches for uveitis. Review of Ophtalmology. 2017; **24**(8):35-39

[35] Criscuolo C, Fabiani C, Cerri E, Domenici L. Synaptic dysfunction in Alzheimer's disease and glaucoma: From common degenerative mechanisms toward neuroprotection. Frontiers in Cellular Neuroscience. 2017;**11**:53. DOI: 10.3389/fncel.2017.00053

[36] Occhiutto ML, Freitas FR, Maranhao RC, Costa VP. Breakdown of the blood-ocular barrier as a strategy for the systemic use of nanosystems. Pharmaceutics. 2012;**4**:252-275. DOI: 10.3390/pharmaceutics4020252

[37] Meshram S, Thorat S. Ocular in situ gels: Development, evaluation and advancements. Scholars Academic Journal of Pharmacy. 2015;**4**(7):340-346

[38] Willermain F, Libert S, Motulsky E, Salik D, Caspers L, Perret J, Delporte C. Origins and consequences of hyperosmolar stress in retinal pigmented epithelial cells. Frontiers in Physiology. 2014;**5**(199):8p. DOI: 10.3389/fphys.2014.00199

[39] Kubo Y, Hosoya K. Inner blood-retinal barrier transporters: Relevance to diabetic retinopathy. In: Diabetic Retinopathy. Vol. 356. 2012. DOI: 10.5772/33992

[40] Gipson IK. The ocular surface: The chalenge to enable and protect vision. Investigative Ophthalmology and Visual Science. Oct 2007;**48**(10):4390-4398. DOI: 10.1167/iovs.07-0770

[41] Conrady CD, Joos ZP, Patel BCK. Review: The lacrimal gland and its role in dry eye. Journal of Ophtalmology. 2016;**2016**:11. Article ID 7542929

[42] Marques MRC, Loebemberg R, Almukainzi M. Simulated biological fluids with possible applications in dissolution testing. Dissolution Technologies. 2011;**18**(3):15-28. DOI: 10.14227/DT180311P15

[43] Schey K, Wang Z, Wenke JL, Qi Y. Aquaporins in the eye: Expression, function and rolesin ocular disease. Biochimica et Biophysica Acta. May 2014;**1840**(5):1513-1523. DOI: 10.1016/j.bbagen.2013.10.037

[44] Yun YH, Lee BK, Garner J. Polysaccharide hydrogels: The present and the future. In: Matricardi P, Alhaique F, Coviello T, editors. Polysaccharide Hydrogels. Characterization and Biomedical Applications. Boca Raton, FL:Taylor & Francis Group; Pan Stanford Publishing; 2016. pp. 499-505

[45] Chitosan: Knowing the History [Internet]. Available from: http://chitosan.in-honolulu.com/ [Accessed: Aug 24, 2017]

[46] Muzzarelli RAA, Mehtedi ME, Bottegoni C, Aquili A, Gigante A. Genipin-crosslinked chitosan gels and scaffolds for tissue engineeringand regeneration of cartilage and bone. Marine Drugs. 2015;**13**:7314-7338. DOI: 10.3390/md13127068

[47] Liu L, Gao Q, Lu X, Zhou H. In situ forming hydrogels based on chitosan for drug delivery and tissue regeneration. Asian Journal of Pharmaceutical Sciences. 2016;**11**:673-683. DOI: 10.1016/j.ajps.2016.07.001

[48] Liskova J, Bacakova L, et al. Development of thermosensitive hydrogels of chitosan, sodium and magnesium glycerophosphate for bone regeneration applications. Journal of Functional Biomaterials. 2015;**6**:192-203

[49] Wang L, Stegemann JP. Thermogelling chitosan and collagen composite hydrogels initiated with β-glycerophosphate for bone tissue engineering. Biomaterials. May 2010; **31**(14):3976-3985. DOI: 10.1016/j.biomaterials.2010.01.131

[50] Chang SH, Lin HTV, Wu GJ, Tsai GJ. pH effects on solubility, zeta potential and correlation between antibacterial activity and molecular weight of chitosan. Carbohydrate Polymers. 2015;**134**:74-81. DOI: 10.1016/j.carbpol.2015.07.072

[51] Chemical Composition of Chitosan Influences Antibacterial Activity [Internet]. 2011. Available from: https://www.news-medical.net/news/20110916/Chemical-composition--of-chitosan-influences-antibacterial-activitY.aspx [Accessed: Oct 1, 2017]

[52] Chitosan Put Forward as Effective Alternative to Growth-promoting Antibiotics in the Diet of Ruminants [Internet]. 2010. Available from: https://www.news-medical.net/

news/20100301/Chitosan-put-forward-as-effective-alternative-to-growth-promoting-antibiotics-in-the-diet-of-ruminants.aspx [Accessed: Oct 1, 2017]

[53] Ribeiro MP, Espiga A. Development of a new chitosan hydrogel for wound healing. Wound Repair and Regeneration. 2009;**17**:817-824. DOI: 10.1111/j.1524-475X.2009.00538

[54] Zhang J, Xia W, Liu P, Cheng Q, Tahirou T, Gu W, Li B. Chitosan modification and pharmaceutical biomedical applications. Marine Drugs. 2010;**8**:1962-1987. DOI: 10.3390/md8071962

[55] Yuan Y, Chesnutt BM, Haggard WO, Bumgardner JD. Deacetyation of chitosan: Material characterization ans in vitro evaluation via albumin adsorption and pre-osteoblastic cell cultures. Materials. 2011;**4**:1399-1416. DOI: 10.3390/ma4081399

[56] Andersen T, Bleber S, Flaten GE, Tho I, Mattsson S, Skalko-Basnet N. Chitosan in mucoadhesive drug delivery: Focus on local vaginal therapy. Marine Drugs. 2015;**13**:222-236. DOI: 10.3390/md13010222

[57] Szymanska E, Winnicka K. Stability of chitosan – A challenge for pharmaceutical and biomedical applications. Marine Drugs. 2015;**13**:1819-1846. DOI: 10.3390/md13041819

[58] Rizwan M, Yahya R, Hassan A. pH sensitive hydrogels in drug delivery: Brief history, properties, swelling and release mechanism, material selection and applications. Polymer. 2017;**9**:137. DOI: 10.3390/polym9040137

[59] Montilla A, Ruiz-Matute AI, Corzo N. Biological effects and extraction processes used to obtain marine chitosan. In: Hernandez-Ledesma B, Herrero M, editors. Bioactive Compounds from Marine Foods. 1st ed. Chichester: Wiley; 2014. pp. 193-210

[60] New Version of Chitosan Effective in Capturing Flu Virus [Internet]. 2011. Available from: https://www.news-medical.net/news/20111103/New-version-of-chitosan-effective-in--capturing-flu-virus.aspx [Accessed: Oct 1, 2017]

[61] Younes I, Rinaudo M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. Marine Drugs. 2015;**13**:1133-1174. DOI: 10.3390/md13031133

[62] Pena A, Sanchez NS, Calahorra M. Effects of chitosan on *Candida albicans*: Conditions for its antifungal activity. BioMed Research International. 2013. Article ID 527549. 15 p. DOI: 10.1155/2013/527549

[63] Walsh AM, Sweeney T, Bahar B, Doherty JV: Multi-functional roles of chitosan as a potential protective agent against obesity. Plos One. 2013;**8**(1)7. DOI: 10.1371/journal.pone.0053828

[64] Zhang Z, Ortiz O, Goyal R, Kohn J. Biodegradable polymers. In: Modjarrad K, Ebnesajjad S, editors. Handbook of Polymer Applications in Medicine and Medical Devices. Kidlington: Elsevier; 2014. p. 313

[65] Gharge V, Pawar P. Recent trends in chitosan based nanotechnology: A reference to ocular drug delivery system. International Journal of Ophtalmology & Virtual Science. 2017;**2**(4):98-105. DOI: 10.11648/j.ijovs.20170204.14

[66] Viens P, Lacombe-Harvey MV, Brrzezinski R. Chitosanase from family 46 of glycoside hydrolases: From proteins to phenotypes. Marine Drugs. 2015;**13**:6566-6587. DOI: 10.3390/md13116566

[67] Azuma K, Osaki T, Minami S, Okamoto Y. Anticancer and anti-inflamatory properties of chitin and chitosan oligosaccharides. Journal of Functional Biomaterials. 2015;**6**(1):33-49. DOI: 10.3390/jfb6010033

[68] Goy RC, de Britto D, Assis OBG. A review of the antimicrobial activity of chitosan. Polimeros.Ciencia e Tecnologia. 2009;**19**(3):241-247. DOI: 10.1590/S0104-14282009000300013

[69] Zhang Y, Huo M, Zhou j YD, Wu Y. Potential of amphiphilically modified low molecular weight chitosan as a novel carrier for hydrophobic anticancer drug: Synthesis, characterization, micellization and cytotoxicity evaluation. Carbohydrate Polymers. 2009;**77**(2):231-238. DOI: 10.1016/j.carbpol.2008.12.034

[70] Shastri DH. Thiolated chitosan: A boon to ocular delivery of therapeutics. Journal of Bioequivalence & Bioavailability 2017;**3**(2):1-5. DOI: 10.15406/mojbb.2017.03.00029

[71] Samuel AJ, Kulkarni M, Tambe R. Thiomers: Forms, features and formulations. Journal of Chemical and Pharmaceutical Research. 2010;**2**(6):316-323

[72] Sreenivas SA, Pai KV. Thiolated chitosans: Novel polymers for mucoadhesive drug delivery – A review. Tropical Journal of Pharmaceutical Research. Sep 2008;**7**(3):1077-1088

[73] Fischak C, Klaus R, Werkmeister RM, Hohenadl C, Prinz M, Schmetterer L, Garhofer G. Effect of topically administered chitosan N-acetylcysteine on corneal wpund healing in a rabbit model. Journal of Ophtalmology. 2017;**2**:1-6. DOI: 10.1155/2017/5192924

[74] Lavik E, Kuehn MH, Kwon YH. Novel drug delivery systems for glaucoma. Eye (London, England). 2011;**25**(5):578-586. DOI: 10.1038/eye.2011.82

[75] Ali Z, Kumar Sharma P, Warsi MH. An insight of natural polymers in ocular drug delivery systems. Journal of Chronotherapy and Drug Delivery. 2016;**7**(1):7-19

[76] Basaran E, Yazan Y. Ocular application of chitosan. Expert Opinion on Drug Delivery. 2012;**9**(6):701-712. DOI: 10.1517/17425247.2012.681775

[77] Aravamudhan A, Nada A, Kumbar S. Natural polymers: Polysaccharides and their derivatives for biomedical applications. In: Kumbar SG, Laurencin CT, Deng M, editors. Natural and Synthetic Biomedical Polymers. 1st. ed. Burlington: Elsevier; 2014. pp. 67-84

[78] Benabid FZ, Zouai F. Natural polymers: Cellulose, chitin, chitosan, g elatin, starch, carrageenan, xylan and dextran. Algerian Journal of Natural Products. 2016;**4**(3):348-357. DOI: 10.5281/zenodo.199036

[79] Kaushik K, Sharma RB, Agarwal S. Natural polymers and their applications. International Journal of Pharmaceutical Sciences Review and Research. 2016;**37**(2):30-36

[80] Felt O, Furrer P, Mayer JM, Plazonnet B, Buri P, Gurny R. Topical use of chitosan in ophtalmology: Tolerance assessment and evaluation of precorneal retention. International Journal of Pharmaceutics. 1999;**180**:185-193. DOI: 10.1016/S0378-5173(99)00003-4

[81] Harikumar SI, Sonia A. Nanotechnological approaches in ophtalmic delivery systems. International Journal of Drug Development & Research. Oct–Dec 2011;**3**(4):9-19

[82] Sahoo S, Sahoo R, Nayak P. Mucoadhesive nanopolymers for posterior segment drug delivery. Retina Today. 2011;**3**:60-63

[83] Wadhwa S, Paliwal R, Paliwal SR, Vyas SP. Nanocarriers in ocular drug delivery: An update review. Current Pharmaceutical Design. 2009;**15**:2724-2750. DOI: 10.2174/138161209788923886

[84] Weng Y, Liu J, Jin S, Guo W, Liang X, Hu Z. Nanotechnology-based strategies for treatment of ocular disease. Acta Pharmaceutica Sinica B. 2017;**7**(3):281-293. DOI: 10.1016/j.apsb.2016.09.001

[85] Scherer D. An integrated pipeline of ophtalmic products based on Eyesol dlivery tchnology. Ophtalmic Drug Delivery. Jan 2015;**54**:21-23

[86] Cholkar K, Vadlapudi AD, Dasari SR, Mitra AK. Ocular Drug delivery. In: Mitra AK, Vadlapudi A, Kwatra D, editors. Drug Delivery. Burlington: Jones & Bartlett Learning; 2014. pp. 219-263

[87] Prajapati BG, Patel MM. Chitosan/PVA bioadhesive ocular inserts of ofloxacin. Drug Development & Delivery. 2012;**12**(5):11

[88] Chaundhari HS, Popat RR, Adhao VS, Shrikhande VN. Dendrimers: Novel carriers for drug delivery. Journal of Applied Pharmaceutical Research. 2016;**4**(1):01-19

[89] Yavuz B, Bozdag Pehlivan S, Unlu N. Dendrimeric systems and their applications in ocular drug delivery. The Scientific World Journal. 2013;**7**: 13 p. DOI: 10.1155/2013/732340

[90] Moosa MR,Choonara YE, du Toit LC, Kumar P, Carmichael T, Tomar LK, Tyagi C, Pillay V. A review of topically administered mini-tablets for drug delivery to the anterior segment of the eye. Advances in Ocular Drug Delivery. 2014;**66**(4):490-506. DOI: 10.1111/jphp.12131

[91] Selvaraj S, Karthikeyan J, Saravanakumar N. Chitosan loaded microspheres as an ocular delivery system for acyclovir. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;**4**(1):125-132

[92] Yuan X, Marcano DC, Shin CS, Hua X, Isenhart LC, Pflugfelder SC, Acharya G. Ocular drug delivery nanowafer with enhanced therapeutic efficacy. ACS Nano. 2015;**9**(2):1749-1758

[93] Rubin AB. A topical ocular ring designed to replace glaucoma eye drops. Opthtalmic Drug Delivery. 2016;**63**:20-21

[94] Muller C, Utkhede D. Therapy without drops: A reality. Ophtalmic Drug Delivery. 2016; **63**:26-27

[95] O'Rourke M :Development of sustained-release ocular delivery technologies Ophtalmic Drug Delivery 2016; 63:4–5

[96] Barman SP. Sustained drug delivery in the posterior segments of the eye. Ophtalmic Drug Delivery. 2015;**54**:26-29

[97] Bibber D. Scalability of micro intraocular implants and device. Ophtalmic Drug Delivery. 2016;**63**:22-24

[98] Thakur RRS, Tekko IA, et al. Rapidly dissolving polymeric microneedles for minimally invasive intraocular drug delivery. Drug Delivery and Translational Research. 2016;**6**: 800-815. DOI: 10.1007/s13346-016-0332-9

[99] Wilson SL, Ahearne M. Hopkinson a:An overview of current techniques for ocular toxicity testing. Toxicology. 2015;**327**:32-46. DOI: 10.1016/j.tox.2014.11.003

[100] ICCVAM Test Method Evaluation Report: Current Validation Status of In Vitro Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products, Appendix B3. 2010; NIH Publication No. 10-7553

[101] Baranowski P, Karolewicz B, Gajda M, Pluta J. Ophtalmic drug dosage forms: Characterisation and research methods. The Scientific World Journal. 2014;**7**:14. DOI: 10.1155/2014/861904

[102] Development and Validation of In Vitro Release Testing Methods for Semisolid Formulations [Internet]. 2009. Available from: http://www.particlesciences.com/news/technical-briefs/2009/in-vitro-release-testing-methods.html [Accessed: Oct 15, 2017]

[103] Kanfer I, Rath S, Purazi P, Mudyahoto NA. In vitro release testing of semi-solid dosage forms. Dissolution Technologies. 2017;**24**(3):52-60. DOI: 10.14227/DT240317P52

[104] Agarwal P, Rupenthal ID. In vitro and ex vivo penetration and absorbtion models. Drug Delivery and Translational Research. 2016;**6**:634-647. DOI: 10.1007/s13346-015-0275-6

[105] Delplace V, Payne S, Shoichet M. Delivery strategies for treatment of age-related ocular d; iseases: From a biological understanding to biomaterial solutions. Journal of Controlled Release. 2015;**219**:652-668. DOI: 10.1016/j.jconrel.2015.09.065

[106] Ahmad FJ, Akhter S, Ahmad ZM, Ramazani F, Samim M, Warsi MH, Anwar M. Prospective corollary of ophtalmic nanomedicine – A concept shift toward chitosan-based mucoadhesive nanomedicine. In: Kim SK, editor. Chitin and Chitosan Derivatives. Advances in Drug Discovery and Developments. Boca Raton: CRC Press, Taylor & Francis Group; 2013. pp. 317-330

[107] Basaran E, Senel BK, Kirimlioglu GY, Yazan Y. Ornidazole incorporated chitosan nanoparticles for ocular application. Latin American Journal of Pharmacy. 2015;**34**(6): 1180-1188

[108] Silva NC, Silva S, Sarmento B, Pintado M. Chitosan nanoparticles for daptomycin delivery in ocular treatment of bacterial endophthalmitis. Drug Delivery. 2015;**22**(7):885-893. DOI: 10.3109/10717544.2013.858195

[109] Shafie MAA, Fayek HHM. Formulation and evaluation of betamethasone sodium phosphate loaded nanoparticles for ophtalmic delivery. Journal of Clinical and Experimental Ophthalmology. 2013;**4**(2):11. DOI: 10.4172/2155-9570.1000273

[110] Selvaraj S, Saravanakumar N, Karthikeyan J, Evangeline D, Lathamary D, Rajendran NN. Acyclovir loaded chitosan nanoparticles for ocular delivery. Der Pharmacia Lettre. 2010;**2**(3):420-431. DOI: 10.4103/0973-8398.76749

[111] Hou D, Gui R, Hu S, Yi H, Feng Z, Ping Q. Preparation and characterization of novel drug-inserted-montmorillonite chitosan carriers for ocular drug delivery. Advances in Nanoparticles. 2015;**4**:70-84. DOI: 10.4236/anp.2015.43009

[112] Ibrahim MM, Abd-Elgawad AEH, Soliman OAE, Jablonski M. Natural bioadhesive biodegradable nanoparticles-based topical ophtalmic formulations for sustained celecoxib release: In vitro study. Journal of Pharmaceutical Technology & Drug Research. 2013:15. DOI: 10.7243/2050-120X-2-7

[113] Vaishya RD, Khurana V, Patel S, Mitra AK. Controlled ocular drug delivery with nanomicelles. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology. Sep 2014;**6**(5):422-437. DOI: 10.1002/wnan.1272

[114] Lin HR, Chang PC. Novel pluronic-chitosan micelle as an ocular delivery system. Journal of Biomedical Materials Research Part B Applied Biomaterials. 2013;**101**(5):689-699. DOI: 10.1002/jbm.b.32871

[115] Somavarapu S, Elsaid Z, Gunic M, Elsaid N, Jackson TL. Amphiphilic chitosan nanomicelles for the topical delivery of rapamycin. Investigative Ophthalmology & Visual Science. 2012;**53**(14):315

[116] Shi S, Zhang Z, Luo Z, Yu J, Liang R, Li X, Chen H. Chitosan grafted methoxi poly (ethylene glycol)-poly (ε-caprolactone) nanosuspension for ocular delivery of hydrophobic diclofenac. Scientific Reports. 2015;**5**:11337. DOI: 10.1038/srep11337

[117] Abdelrahman AA, Salem HF, Khallaf RA, Ali AMA. Modeling, optimization and in vitro corneal permeation of chitosan-lomefloxacin HCl nanosuspension intended for ophtalmic delivery. Journal of Pharmaceutical Innovation. 2015;**10**(3):254-268. DOI: 10.1007/s12247-015-9224-7

[118] Ahuja M, Verma P, Bhatia M. Preparation and evaluation of chitosan-itraconazole co-precipitated nanosuspension for ocular delivery. Journal of Experimental Nanoscience. 2015;**10**(3):209-221. DOI: 10.1080/17458080.2013.822108

[119] Mishra GP, Bagui M, Tamboli V, Mitra AK. Recent applications of liposomes in ophtalmic drug delivery. Journal of Drug Delivery. 2011. Article ID 863734. 14 p. DOI: 10.1155/2011/863734

[120] Tsukamoto T, Hironaka K, Fujisawa T, Yamaguchi D, Tahara K, Tozuka Y, Takeuchi H. Preparation of bromfenac-loaded liposomes modified with chitosan for ophtalmic drug delivery and evaluation of physiochemical properties and drug release profile. Asian Journal of Pharmaceutical Sciences. 2013;**8**:104-109. DOI: 10.1016/j.ajps.2013.07.013

[121] Li N, Zhuang CY, Wang M, Sui CG, Pan WS. Low molecular weight chitosan-coated liposomes for ocular drug delivery: In vitro and in vivo studies. Drug Delivery. 2012; **19**(1):28-35. DOI: 10.3109/10717544.2011.621994

[122] Ustundag-Okur N, Gokce EH, Bozbiyik DI, Egrilmez S, Ozer O, Ertan G. Preparation and in vitro-in vivo evaluation of ofloxacin loaded ophtalmic nano structured lipid carriers modified with chitosan oligosaccharide lactate for the treatment of bacterial keratitis. European Journal of Pharmaceutical Sciences. 2014;**63**:204-215. DOI: 10.1016/j.ejps.2014.07.013

[123] Hegde RR, Verma A, Ghosh A. Microemulsion: New insights into the ocular drug delivery. ISRN Pharmaceutics. 2013;**2013**:11. Article ID 826798. DOI: 10.1155/2013/826798

[124] Bhosale R, Bhandwalkar O, Duduskar A, Jadhav R, Pawar P. Water soluble chitosan mediated voriconazole microemulsion as sustained carrier for ophtalmic application: In vitro/ex vivo evaluations. Open Pharmaceutical Sciences Journal. 2016;**3**:215-234. DOI: 10.2174/1874844901603010215

[125] Yamaguchi M, Ueda K, Isowaki A, Ohtori A, Takeuchi H, Ohguro N, Tojo K. Mucoadhesive properties of chitosn-coated ophtalmic lipid emulsion containing indomethacin in tear fluid. Biological & Pharmaceutical Bulletin. 2009;**32**(7):1266-1271. DOI: 10.1248/bpb.32.1266

[126] Mitra A, Dey B. Chitosan microspheres in novel drug delivery systems. Indian Journal of Pharmaceutical Sciences. Jul–Aug 2011;**73**(4):355-366. DOI; 10.4103/0250-474X.95607

[127] Kapanigowda UG, Nagaraja SH, Ramaiah B, Boggarapu PR. Improved intraocular bioavailability of ganciclovir by mucoadhesive polymer based ocular microspheres: Development and simulation process in Wistar rats. DARU Journal of Pharmaceutical Sciences. 2015;**23**:49. DOI: 10.1186/s40199-015-0132-7

[128] Rajawat GS, Shinde UA, Nair HA. Chitosan-N-acetyl cysteine microspheres for ocular delivery of acyclovir: Synthesis and in vitro/in vivo evaluation. Journal of Drug Delivery Science and Technology. Oct 2016;**35**:333-342. DOI: 10.1016/j.jddst.2016.08.006

[129] Patrianakos TD. Optimizing the efficacy of topical medications. Review of Ophtalmology. 2017;**24**(10):52-54

[130] Mateescu MA, Ispas-Szabo P, Assaad E: Chitosan and its derivatives as self-assembled systems for drug delivery. In: Mateescu MA, Ispas-Szabo P, Assaad E, editors. Controlled Drug Delivery. 1st ed. Cambridge: Woodhead Publishing Limited; 2015. pp. 86-119

[131] Jain D, Kumar V, Singh S, Mulletz A, Bar-Shalom D. Newer trends in in situ gelling systems for controlled ocular drug delivery. Journal of Analytical & Pharmaceutical Research. 2016;**2**(3):00022. DOI: 10.15406/japlr.2016.02.00022

[132] Chavan C, Bala P, Pal K, Kale SN. Cross-linked chitosan dextran sulphate vehicle system for controlled release of ciprofloxacin drug: An ophtalmic application. OpenNano. 2017; **2**:28-36. DOI: 10.1016/j.onano.2017.04.002

[133] Varshosaz J, Tabbakhian M, Sulmani Z. Designing of a thermosensitive chitosan/ poloxamer in situ gel for ocular delivery of ciprofloxacin. The Open Drug Delivery Journal. 2008;**2**:61-70

[134] Gupta S, Vyas SP. Carbopol/chitosan based pH triggered in situ gelling system for ocular delivery of timolol maleate. Scientia Pharmaceutica. 2010;**78**(4):959-976. DOI: 10.3797/ scipharm.1001-06

[135] Zaki R, Hosny KM, Khames A, Abd-elbary A. Ketorolac tromethamine in-situ ocular hyrogel: Preparation, characterization and in-vivo evaluation. International Journal of Drug Delivery. 2011;**3**(3):535-545

[136] Gilhotra RM, Mishra DN. Failure of calcium gluconate internal gelation for prolonging drug release from alginate-chitosan-based ocular insert of atenolol. Journal of Pharmaceutical Negative Results. 2010;**1**(2):35-39. DOI: 10.4103/0976-9234.75703

[137] Li W, Long Y, Liu Y, Long K, Liu S, Wang Y, Ren L. Fabrication and characterization of chitosan-collagen crosslinked membranes for corneal tissue engineering. Journal of Biomaterials Science, Polymer Edition. 2014;**25**(17):1962-1972. DOI: 10.1080/09205063.2014.965996

[138] Fabiano A, Bizzarri R, Zambito Y. Thermosensitive hydrogel based on chitosan and its derivatives containing medicated nanoparticles for transcorneal administration of 5-fluorouracil. International Journal of Nanomedicine. 2017;**12**:633-643. DOI: 10.2147/IJN.S121642

[139] Ashton P. Huge therapeutic advances: Bigger drug delivery opportunities. Opthalmic Drug Delivery. 2015;**54**:4-6

[140] Ashton P. pSvida and ophtalmic drug delivery. Ophtalmic Drug Delivery. 2015;**54**:18-19

[141] Garg VK, Garg G. Ocular inserts – Advancement in therapy of eye diseases. Journal of Advanced Pharmaceutical Technology & Research. Jul–Sep 2010;**1**(3):291-296. DOI: 10. 4103/0110-5558.72419

[142] De Souza JF, Nunes Maia K : Ocular inserts based on chitosan and brimonidine tartrate: Development, characterization and biocompatibility. Journal of Drug Delivery Science and Technology.2016;**32**:21-30. DOI: 10.1016/j.jddst.2016.01.008

[143] Foureaux G, Franca JR, Nogueira JC, Fulgêncio Gde O, Ribeiro TG, Castilho RO, Yoshida MI, Fuscaldi LL, Fernandes SO, Cardoso VN, Cronemberger S, Faraco AA, Ferreira AJ. Ocular inserts for sustained release of the angiotensin-converting enzyme 2 activator, diminazene aceturate to treat glaucoma in rats. PLoS One. Jul 23, 2015;**10**(7):18. DOI: 10.1371/journal.pone.0133149

[144] Upadhyaya N, Patidar A, Agrawal S, Gupta D. Development and evaluation of polymeric sustained release levofloxacin ocuserts. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2011;**2**(3):411-420

[145] Franca JR, Foureaux G, Fuscaldi LL, Ribeiro TG, Rodrigues LB, Bravo R, Castilho RO, Yoshida MI, Cardoso VN, Fernandes SO, Cronemberger S, Ferreira AJ, Faraco AA: Bimatoprost-loaded ocular inserts as sustained release drug delivery systems for glaucoma tratment: In vitro and in vivo evaluation. Plos One. 2014;**9**(4):11. DOI: 0.1371/journal.pone.009546

[146] Hu X, Hao L, Wang H, Yang X, Zhang G,Wang G, Zhang X. Hydrogel contact lens for extended delivery of ophtalmic drugs. International Journal of Polymer Science. 2011. Article ID ID814163. 9 p. DOI: 10.1155/2011/814163

[147] Carvalho LM, Marques CS, Oliveira RS, Coelho PB, Costa PC, Ferreira DC. Sustained drug release by contact lenses for glaucoma treatment- a review. Journal of Controlled Release. 2015;**202**:76-82. DOI: 10.1016/j.jconrel.2015.01.023

[148] ElShaer A, Mustafa S, Kasar M, Thapa S, Ghatora B, Alany RG. Nanoparticle-laden contact lens for controlled ocular delivery of prednisolone: Formulation, optimization using statistical experimental design. Pharmaceutics. 2016;**8**:14. DOI: 10.3390/pharmaceutics 8020014

[149] Pimenta AFR, Valente A, Pereira JMC, Pereira JCF, Filipe HP, Mata JIG, Colaco R, Sramago B, Serro AP. Simulation of the hydrodynamic conditions of the eye to better reproduce the drug release from hydrogel contact lenses: Experiments and modelling. Drug Delivery and Translational Research. 2016;**6**:755-762

[150] Sabzevar FT, Mohajeri SA. Development of ocular drug delivery systems using molecularly imprinted soft contact lenses. Drug Development and Industrial Pharmacy. May 2015;**41**(5):703-713. DOI: 10.3109/03639045.2014.948451

[151] Behl G, Iqbal J, O'Relly NJ, McLoughlin P, Fitzhenry L. Synthesis and characterization of poli(2-hydroxyethylmethacrylate) contact lenses containing chitosan nanoparticles as an ocular drug delivery system for dexamethasone sodium phosphate. Pharmaceutical Research. 2016;**33**(7):1638-1648. DOI: 10.1007/sl 1095-016-1903-7

[152] Xin-Yuan S, Tian-Wei T. New contact lens based on chitosan/gelatin composites. Journal of Bioactive and Compatible Polymers. 2004;**19**(6):467-479. DOI: 10.1177/0883911504048410

[153] Hu XH, Tan HP, Li D, Gu MY. Surface functionalisation of contact lenses by CS/HA multilayer film to improve its properties and deliver drugs. Materials Technology-Advanced Performance Materials. 2014;**29**(1):8-13. DOI: 10.1179/1753555713Y.0000000063

[154] Shivaji DP, Ganesh PD, Rhanudas SR. Formulation and characterization of ocular minitablets for controlled drug delivery of fluoroquinolones. World Journal of Pharmacy and Pharmaceutical Sciences. 2014;**3**(8):1467-1482

[155] Udawant SV, Gondkar SB, Saudagar RB. A review: Topically administered ocular minitablets. International Journal of Institutional Pharmacy and Life Sciences. 2015;**5**(5):212-230

[156] EL-Gawad A, Soliman OA, Barker SA, Girgis GNS. Formulation and evaluation of gel forming ocular minitablets containing piroxicam. British Journal of Pharmaceutical Research. 2012;**2**(3):141-167. DOI: 10.9734/BJPR/2014/1653

[157] Refai H, Tag R. Development and characterization of sponge-like acyclovir ocular minitablets. Drug Delivery. 2011;**18**(1):38-45. DOI: 10.3109/10717544.2010.509364

[158] Verestiuc L, Nastasescu O, Barbu E, Sarvaiya I, Green KL, Tsibouklis J. Functionalized chitosan/NIPAM (HEMA) hybrid polymer networks as inserts for ocular drug delivery: Synthesis, in vitro assessment, and in vivo evaluation. Journal of Biomedical Materials Research Part A. 2006;**77**(4):726-735. DOI: 10.1002/jbm.a.30668

[159] Gilhotr RM, Gilhotra N, Mishra DN. A hydrogel–forming bioadhesive ocular minitablet for the management of microbial keratitis. Bioadhesive ocular minitablet/Asian Journal of Pharmaceutical Sciences. 2010;**5**(1):19-25