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Nanoparticles for drug delivery to the anterior segment of the eye

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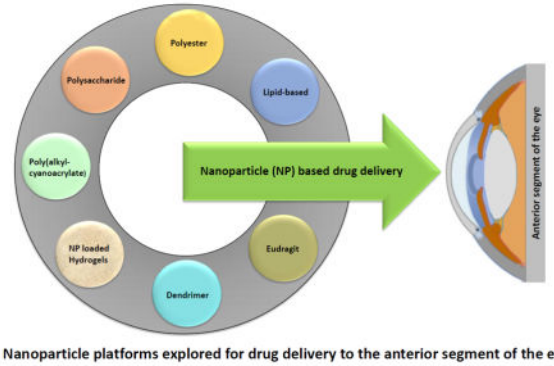
Abstract

Commercially available ocular drug delivery systems are effective but less efficacious to manage diseases/disorders of the anterior segment of the eye. Recent advances in nanotechnology and molecular biology offer a great opportunity for efficacious ocular drug delivery for the treatments of anterior segment diseases/disorders. Nanoparticles have been designed for preparing eye drops or injectable solutions to surmount ocular obstacles faced after administration. Better drug pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, and biorecognition can be achieved to improve drug efficacy when drugs are loaded in the nanoparticles. Despite the fact that a number of review articles have been published at various points in the past regarding nanoparticles for drug delivery, there is not a review yet focusing on the development of nanoparticles for ocular drug delivery to the anterior segment of the eye. This review fills in the gap and summarizes the development of nanoparticles as drug carriers for improving the penetration and bioavailability of drugs to the anterior segment of the eye.

Graphical Abstract



Nanoparticle platforms explored for drug delivery to the anterior segment of the eye

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Keywords

nanoparticles; anterior segment of the eye; ocular barriers; poly(alkyl cyanoacrylate); polysaccharide; polyester; EUDRAGIT®; lipid; dendrimer; contact lenses

1. Introduction

The eye is a delicate and very complicated organ having two main anatomical segments: the anterior segment and the posterior segment (Figure 1). The anterior segment is a part of the eyeball that is anterior to the lens and mainly consists of the cornea, conjunctiva, iris, lens, ciliary body, and the anterior portion of the sclera. It is further divided into two chambers, the anterior (between the posterior surface of the cornea and iris) and posterior (between the posterior surface of the cornea and the iris) chambers, which are connected by the opening of the pupil and filled with aqueous humor secreted by ciliary processes. The aqueous humor provides nutrients for lens and cornea, maintains intraocular pressure, and is replaced several times a day [1]. The diseases/disorders occurring in the anterior segment include cataracts, dry eye, congenital and developmental abnormalities, inflammatory diseases, infectious diseases, hereditary and degenerative diseases, glaucoma, tumors, injury, trauma, and ocular manifestations of systemic diseases. Clinically, these anterior segment diseases/disorders are more often treated by using eye drops or ointments. However, the efficacy of the eye drop and ointment treatments is low due to the existence of ocular barriers including the cornea and conjunctival barriers covering the ocular surface, and tear drainage (Figure 1) [1, 2]. Nevertheless, local therapy via topical and periocular administrations is more favorable over therapies via systemic administration such as oral and intravenous administrations to manage ocular diseases. The first reason is that the eye has much fewer blood vessels and less blood flow than the whole body blood circulatory system so that the amount and rate of drugs to be cleared through local eye administration is much less than through systemic administration. The second reason is due to the presence of blood-aqueous barrier, which limits the drug penetration from the systemic circulation into the anterior segment of the eye. Recent advances in nanoparticles offer a great opportunity for efficient local delivery of drugs to the anterior segment. The advantages of nanoparticles include enhancing drug permeability across the blood-aqueous barrier and cornea, prolonging drug contact time with ocular tissues, delivering drugs to a specific tissue site in a controlled manner, protecting drugs from degradation and metabolism to enhance drug stability, sustaining drug release for weeks to months, having low to no toxicity and side effects, maintaining long shelf-life, and needing no reconstitution and no surgical removal. In this review, we will discuss the routes of administration and ocular barriers and summarize the up-to-date development of polymeric nanoparticles for drug delivery to the anterior segment of the eye.

2. Routes of administration and ocular barriers for delivering drugs to the anterior segment

There are four routes of administration for drugs to reach the anterior segment of the eye: topical, intracameral, subconjunctival, and systemic routes. Depending on the routes of administration, one or more ocular barriers need to be circumvented for drugs to reach the

disease/action sites in the anterior segment of the eye. Table 1 lists the four routes of administration and their associated advantages and limitations. Figure 1 illustrates the structure of the anterior segment of the eye and ocular barriers for drug delivery. In the following, we will discuss the details of the four routes of administration and related ocular barriers.

Topical administration is the simplest, most convenient, self-administrable, non-invasive, and most dominant drug administration route for the management of anterior segment diseases/disorders. It is a local drug delivery method, avoiding the blood–aqueous barrier, and the side effects and first-pass metabolism that may occur in some systemically administrated drugs. Drugs administrated through the topical route are usually formulated into eye drops. Depending on the formulation and the drugs' physiochemical characteristics, drugs can reach various external (cornea, conjunctiva sclera) and internal (iris, ciliary body, aqueous humor, vitreous humor, retina) sites in the eye after topical instillation (Figure 2) [3, 4]. However, only 1–7% of the administered drugs can reach the aqueous humor due to the tear film, and cornea and conjunctiva barriers [5–7]. The tear film is the first obstacle faced for topically administered drugs. It consists of three layers: an outermost lipid layer, a thicker aqueous middle layer and an innermost mucin layer (Figure 1). The tear film is created by tears which are composed of water, electrolytes, and many different proteins that work together to promote healing and fight infection. A human tear has a total volume of about 7~30 μL with a turnover rate of 0.5~2.2 μL/min, and tear film has a rapid restoration time of 2~3 min [8]. Due to the fast turnover rate and time of tear film, the topically administered eye drops are quickly washed away and drained into nasolacrimal duct after instillation. The cornea is the second ocular barrier limiting the penetration of exogenous substances into the eye. It is composed of five layers: epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium (Figure 1). The layers which form substantial barriers to drug penetration are epithelium, stroma, and endothelium. The superficial corneal epithelium is composed of multiple layers of stratified squamous non-keratinized epithelial cells. It limits the permeation of hydrophilic drugs through the cornea due to the hydrophobicity of the epithelium and the presence of tight junctional proteins between the corneal epithelial cells [3, 9]. The inner layer next to the corneal epithelium is the stroma. The stroma is comprised of glycosaminoglycans and collagen fibrils in lamellar structures, and it has a hydrophilic environment. It restricts the penetration of lipophilic drugs through the cornea [3]. The innermost layer of the cornea is monolayer corneal endothelium composed of hexagonal-shaped endothelial cells. This corneal endothelium is leakier than the epithelium and allows the penetration of macromolecules into the aqueous humor on the other side [3]. Overall, the cornea acts as a physical barrier to hydrophilic drugs due to the superficial corneal epithelial layers, and to lipophilic drugs due to the stroma. Besides the cornea route, topically administered drugs can be absorbed into the anterior segment through a non-cornea route: the conjunctiva/sclera pathway, as the conjunctiva has a larger surface area than the cornea (17 vs. ~ 1 cm^2) [5, 6, 10, 11]. Conjunctiva is a rate-limiting barrier for permeation of water-soluble drugs [12, 13] due to rapid drug elimination by conjunctival blood and lymphatic flow. After escaping from conjunctival elimination, drugs penetrate through the sclera to reach the anterior segment (trans-scleral pathway). The sclera has large surface area and relatively high permeability

than the cornea, and the trans-scleral permeation mainly depends on the size of the drug molecules rather than their lipophilicity [3, 14, 15].

Intracameral administration is a method for direct injection of drugs into the anterior chamber in the anterior segment of the eye [16]. It is a local drug delivery method, avoiding the side effects and the first-pass metabolism that may occur in some systemically administrated drugs. It also avoids the cornea, conjunctiva, and blood-aqueous barriers. Therefore, intracameral injection can deliver drugs to the anterior segment relatively easily with high efficiency and was expected to achieve 300 to 600 times more aqueous humor drug level than topical application [17, 18]. After intracameral injection, the mechanism for drugs to reach tissues in the anterior segment is via diffusion and the bulk flow of aqueous humor. In general, the literature supports the safety of intracamerally injected antibiotics such as vancomycin, moxifloxacin, and cephalosporins [18–20]. In addition, the intracameral injection technique was shown to be useful in the off-label use of anti-vascular endothelial growth (anti-VEGF) agents and triamcinolone acetonide for the management of neovascularization in the treatment of neovascular glaucoma and endothelial allograft rejection after penetrating keratoplasty, respectively [21, 22]. However, intracameral antibiotics usually need reconstitution including dilution and other special preparations which require sterilization, preservative-free, and proper concentration and dose. If incorrect dosing and preparation occur, corneal endothelial toxicity and toxic anterior segment syndrome become big concerns [18, 20].

Subconjunctival administration places drugs into the subconjunctival space around the outside of the sclera, and then drugs penetrate through the sclera and reach the anterior segment. It is a minimally invasive and effective route for delivering drugs to the anterior segment, avoiding the cornea and blood–aqueous barriers, and the side effects and first-pass metabolism that may occur in some systemically administrated drugs. It is a local drug delivery method, and can offer sustained drug delivery depending on formulations or devices. However, the subconjunctival route has the limitation of possible loss of drugs to the systemic circulation due to the drainage via the conjunctival blood and lymphatic vessels [14, 23].

Systemic administration can deliver drugs to the anterior segment, but with low bioavailability for conventional ophthalmic formulations such as solutions and suspensions, due to the presence of the blood–aqueous barrier. The two layers that comprise the blood-aqueous barrier (BAB) are the endothelium of the iris/ciliary blood vessels and the nonpigmented epithelium of the ciliary body (Figure 1) [1, 3]. Because of the presence of the tight junctional complexes in both these layers, BAB restricts the penetration of drug molecules from the blood into the aqueous humor [3, 24]. In order to achieve therapeutic levels of drugs in the aqueous humor, high doses are necessary, and high doses can cause adverse systemic side effects so that the systemic administration route is rarely used to treat anterior segment disease/disorders. For clarification, we would like to specifically mention that the term bioavailability in this review means the rate and extent of drug available at the target anterior ocular site such as tear fluid, corneal or conjunctival tissue, aqueous humor, iris, *etc.* after drug administration [25–28]. Three pharmacokinetics (PK) parameters that are commonly used for assessing ocular bioavailability are: C_{max}, the maximum drug

concentration achieved at target ocular site; T_{max}, the time required to achieve the C_{max}; and *AUC*, the area under curve (drug concentration in ocular tissue vs time curve) [27, 29–31]. *AUC* is the most commonly used parameter to describe ocular bioavailability [30], and noted as $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}\infty)}$ for time 0 to *t* and 0 to infinity, respectively [31].

3. Nanoparticles for drug delivery to the anterior segment

As discussed above, the ocular bioavailability of topically administrated drugs is limited due to several factors such as small anatomical space, baseline and reflex lachrymation tear drainage, metabolic degradation of the drugs, and other anatomical and physiological barriers in the eye [7, 24, 32–35]. Appropriate concentrations of the drug are needed at targeted sites in the eye to achieve therapeutic effects of the drugs. To obtain the appropriate concentrations, frequent instillation of eye drops containing the drugs is the common method. However, the frequent dosing method can cause toxic side effects and damage to ocular tissues [3]. It is important and necessary to develop technologies that can deliver drugs to the targeted sites in the eye with low or no frequent dosing. Therapeutic targets for drugs to treat anterior eye segment - related diseases can be located in extra- or intraocular tissues [36]. Based on the therapeutic target areas, the goals of drug delivery to the anterior segment of the eye can be sub-divided into two categories, a) to improve the bioavailability the drugs at extraocular tissues to alleviate the signs and symptoms caused by ocular surface inflammatory disorders of cornea and conjunctiva, such as dry eye syndrome and allergic diseases; and b) to enhance the bioavailability of the drugs in the intraocular tissues to treat infections and complex, vision-threatening diseases such as glaucoma or intraocular inflammation (uveitis). Nanoparticles have large surface areas and can be made of many types of materials with multifunctional surface groups. These materials hold great promise in helping drugs to reach targeted sites to achieve the stated two purposes of drug delivery to the anterior segment of the eye [2, 37–44]. The reasons are that nanoparticles can be entrapped in the ocular mucus layer and then bioadhesive polymer chains/materials that form the nanoparticles can intimately interact with the extraocular tissues to prolong the residence time of drugs loaded in the nanoparticles and reduce drug drainage and thus improve the bioavailability the drugs at extraocular tissues [33]. Furthermore, nanoparticles can also penetrate through ocular surface tissues to deliver encapsulated drugs to intraocular tissues so that improved bioavailability of the drugs at the intraocular tissues can be achieved [45]. The penetration depends on the size, charge, architecture, surface chemistry, and hydrophilicity/hydrophobicity of the nanoparticulate systems [46–48]. Table 2 summarizes some of the important characteristics/properties of the nanoparticles and corresponding characterization techniques used to determine those properties.

The penetration mechanisms for nanoparticles into the interior segment of the eye have not been deeply studied; however, the commonly accepted endocytosis mechanism in explaining nanoparticles' tissue penetration was also observed by Robinson and his colleagues when they used fluorescence microscopy to study the penetration of poly(butyl cyanoacrylate) nanoparticles across the *ex vivo* rabbit cornea and conjunctiva [46, 49–54]. The most commonly used *in vitro* and *ex vivo* models for evaluating the corneal penetration have been summarized by Agarwal *et al* [55]. Additionally, nanoparticles can protect drugs from

degradation and sustain release of drugs which can also increase the bioavailability of the drugs in both extra- and intraocular tissues.

In the following discussions, we will systematically review the progress and challenges in the development of polymeric nanoparticles and their composites with hydrogel matrix for delivering drugs to the anterior segment. These polymeric nanoparticles are made of poly(alkyl cyanoacrylate), polysaccharides, polyesters, EUDRAGIT® polymers and lipids, whose chemical structures are illustrated in Tables 2–5. There are other strategies such as imprinted contact lenses [56], vitamin E based contact lenses [57], and nanowafer-based systems [58–61] that have been used for extended ocular drug delivery to the anterior segment; however, these strategies are not discussed in this review as the main focus of this review is nanoparticles rather than other systems for drug delivery to the anterior segment.

3.1. Poly(alkyl cyanoacrylate) nanoparticles

Poly(alkyl cyanoacrylate) (PACA, Table 2) nanoparticles emerged in the early 1980s and have been extensively studied for drug delivery to the brain and eye [62]. PACA nanoparticles were prepared through emulsion and interfacial polymerizations from monomers, or nanoprecipitation and emulsion-solvent evaporation from presynthesized PACA polymers [62]. Due to the excellent adhesive properties of alkyl cyanoacrylates, PACA nanoparticles were the first nanoparticles investigated for topical ocular drug delivery to the eye [54, 63–68]. Poly(2-hexyl cyanoacrylate) nanoparticles were found to have 4-fold greater accumulation in inflamed albino rabbit eye tissues than healthy tissue. [63, 66]. Hydrophobic drugs such as pilocarpine [64] and acyclovir [69], and hydrophilic drugs such as betaxolol hydrochloride [67] and amikacin sulfate [68], were successfully loaded into PACA nanoparticles. Poly(butyl cyanoacrylate) PBCA nanoparticles improved myotic response to pilocarpine in albino rabbits [64]; increased the concentration of amikacin sulfate in the cornea and aqueous humor in the presence of dextran 70000 [68]; and enhanced the absorption of positively charged betaxolol hydrochloride with decreasing its surface zeta potential and the subsequent antiglaucoma activity of betaxolol hydrochloride, depending on the amount of the drug released [67]. Table 6 summarizes various drug-loaded PACA nanoparticles and their characteristics. Since PACA nanoparticles were found to cause cell lysis and cornea damage in 90’s [54, 62], PACA nanoparticles have been seldom investigated for ocular drug delivery although improved ocular toleration of PACA nanoparticles was observed in the six-hour tolerability study by coating PACA nanoparticles using poly(ethylene glycol) (PEG) polymers [69]. Researchers switched to other more biocompatible polymers for the development of polymeric nanoparticles for ocular delivery as discussed below.

3.2. Polysaccharide nanoparticles

Polysaccharides are long chain carbohydrate molecules having a large number of reactive functional groups varying in chemical composition. Their molecular weights vary widely due to the diversity of their structures and properties. Common hydrophilic functional groups present in the polysaccharides are hydroxyl, carboxyl and amino groups. These functional groups can form hydrogen bonds with mucosa to become mucoadhesive [70]. Due to the hydrogen bonds, when mucoadhesive polysaccharides are added in the eye drops,

the drug's retention time in the tear film and anterior chamber is prolonged, and the clearance of the drug is controlled by the mucus turnover rate rather than the tear turnover rate and is much slower [33, 71–78]. Besides the mucoadhesive property, nature-originated polysaccharides such as chitosan and its derivatives, alginate, hyaluronic acid, gum cordia, and carboxymethyl tamarind kernel polysaccharide (Table 3), possess other appealing properties such as acceptable biocompatibility, excellent ocular tolerance [79, 80], biodegradability [81], and ability to enhance drug membrane permeability both *in vitro* [82] and *in vivo* [83]. The combination of these properties makes polysaccharides versatile biopolymers for ocular drug delivery [71, 84–89]. Drugs such as cyclosporine A (CsA) [90], dorzolamide hydrochloride [91, 92], pramipexole hydrochloride [91], acyclovir [93], 5-flurouracil [94], carteolol [92], gatifloxacin [95], betamethasone sodium phosphate [96], gene [97], pilocarpine [98], econazole nitrate [99], natamycin [100], daptomycin [89], amphotericin B [101], celecoxib [102], timolol maleate [103, 104], fluconazole [105], sodium diclofenac [106], and tropicamide [107] were loaded into nanoparticles made of polysaccharides for ocular delivery. The release of the drugs from these hydrophilic polymer nanoparticle matrices relies on various factors such as polymer hydration, solvent penetration, drug diffusion, drug dissolution, and/or polymer erosion [95]. The discussions below detail the studies carried out on polysaccharide-based nanoparticles for drug delivery to the anterior segment of the eye.

3.2.1. Chitosan nanoparticles—Chitosan has arisen as a promising material for the improvement of drug delivery to the ocular mucosa and is most extensively studied polysaccharide. Chitosan is a cationic linear polysaccharide copolymer of 1,4-(2-amino-2-deoxy-*D*-glucopyranose) and 1,4-(2-acetamido-2-deoxy-*D*-glucopyranose). The mucoadhesive nature of chitosan is related to the interaction between the positively charged amino groups of chitosan and the negatively charged residues of sialic acid in the mucus of cornea and conjunctiva [108] along with other interactions such as hydrogen bonds [109]. Another characteristic of chitosan is its penetration enhancement properties, as it can transiently open the tight junctions between cells [85, 110]. Chitosan nanoparticles are often synthesized via ionic gelation techniques using sodium tripolyphosphate as a crosslinker [80, 85, 86, 111, 112]. The benefits associated with chitosan-based nanoparticles include their ability to contact intimately with the corneal and conjunctival surfaces, and thus enhance drug delivery to external ocular tissues while minimizing the toxicity of the drugs to the internal ocular tissues and blood stream (through systemic absorption). Therefore, the chitosan-based nanoparticles show promising potential for the treatment of external ocular diseases [90]. Table 7 summarizes various drug-loaded chitosan nanoparticles and their characteristics.

Alonso and associates first reported the study of exploring chitosan nanoparticles for delivery of drugs to the ocular surface in 2001 [90] and subsequently conducted extensive studies on chitosan nanoparticles [80, 90, 97, 112]. Synthesized chitosan nanoparticles were reported to have an average size of several hundred nanometers depending on the synthetic conditions, and an almost constant zeta potential of about +35 to +37 mV [80, 90, 93, 102, 112] which was higher than +30 mV implying that the nanoparticles were stable due to the prevention of the aggregations of the nanoparticles by the surface charges [93]. *In vitro*

studies of chitosan nanoparticles in simulated fluids containing mucus components (mucin and lysozyme) showed that the presence of lysozyme did not compromise the stability of chitosan nanoparticles in the tear fluid. The interaction of chitosan nanoparticles with mucin did not significantly affect the viscosity of the mucin dispersion [112]. These chitosan nanoparticles were not inherently toxic to Chang conjunctival cell line at concentrations up to 2 $mg{\cdot}ml^{-1}$ (Figure 3) [112] and spontaneously immortalized epithelial cell line from normal human conjunctiva (IOBA-NHC) at concentrations up to 1 $mg{\cdot}ml^{-1}$ for up to 24 h [80]. *In vivo* examination of topically administrated chitosan nanoparticles in rabbits showed that the nanoparticles did not cause inflammation, tissue alteration, and damage to the epithelial layer of the rabbit cornea as shown in Figure 4 [80, 94]. Another *in vivo* investigation revealed that fluorescein-containing chitosan nanoparticles significantly accumulated in the rabbit cornea and conjunctiva after topically instilled to the *cul-de-sac* of conscious rabbits, evidenced by fluorescence intensity measurements and confocal image (Figures 5 and 6) [112] [80]. Further study of chitosan nanoparticles as a drug carrier for cyclosporine A (CsA) revealed that the nanoparticles showed a high *in vitro* burst release of the drug CsA (62% within 15 min) in sink conditions due to the rapid dissolutions of both the hydrophilic chitosan nanoparticles and the drug present at or close to the surface of the nanoparticles. But the authors speculated that the burst release would be minimized *in vivo* due to the absence of such a significant dilution process upon administration [90]. *In vivo* investigation of the ocular disposition of CsA showed that the therapeutic concentrations of CsA were attained in the external ocular tissues (cornea and conjunctiva) within 48 h after topical instillation of CsA-loaded chitosan nanoparticles in rabbits, while the CsA levels in the inner ocular structures (iris/ciliary body and aqueous humor), blood and plasma were negligible or undetectable. The therapeutic concentration levels in the external ocular tissues were found to be significantly higher after the instillation of CsA-loaded chitosan nanoparticles compared with the control CsA-containing chitosan (not chitosan nanoparticle) solution and aqueous CsA suspension [90]. These results demonstrated that chitosan nanoparticles could be used as carriers to enhance the therapeutic index of clinically challenging drugs such as CsA with the potential application at the extraocular level. Besides CsA, chitosan nanoparticles have also been studied for ocular delivery of other drugs such as dorzolamide hydrochloride [91, 92], pramipexole hydrochloride [91], acyclovir [93], 5-flurouracil [94], carteolol [113], and ketorolac tromethamine [114]. The *in vitro* release profiles of the drugs dorzolamide hydrochloride, pramipexole hydrochloride, acyclovir, 5-flurouracil, and carteolol showed a high burst release [91, 93, 94] as that of CsA [90]. The mucoadhesive strength of dorzolamide hydrochloride-, and pramipexole hydrochloride-loaded chitosan nanoparticles decreased with increasing the drug content due to the decreased amount of chitosan that was available for adhesion and the increased size of the nanoparticles [91]. Topically instilled 5-fluorouracil-loaded chitosan nanoparticles exhibited significantly higher maximum concentration C_{max} (~2.7 times) and bioavailability (AUC ~3.5 times during 8 h) of fluorouracil in the aqueous humor of rabbits when compared with the drug solution alone [94]. Whole body images by gamma scintigraphy revealed that chitosan nanoparticles prolonged the retention of cateolol on the corneal and conjunctival surfaces of rabbits to significantly enhance its ocular hypotensive effect in 24 h study [92].

3.2.2. Chitosan-based hybrid nanoparticles—Due to the hydrophilic nature of chitosan, nanoparticles made of chitosan alone have limited encapsulation potential for hydrophobic drugs [115]. To further improve the drug entrapment, as well as specific targeting, controlled release and toxicity reduction capability of nanoparticles made of chitosan alone, a second polymer or oligomer such as sodium alginate, hyaluronic acid, cholesteryl 3-hemisuccinate, dextran sulfate, sulfobutyl ether-cyclodextrin, Carbopol®, or lecithin (Tables 3 and 4) has been added into the chitosan nanoparticles for ocular drug delivery applications [95, 99, 101, 103, 115–120]. These second polymers/oligomers have plenty of anionic carboxylic, sulfonate or phosphate groups which can form ionic interaction with cationic chitosan so that they can act as crosslinkers like tripolyphosphate which is commonly used for crosslinking chitosan. The crosslinking can increase the tightness of chitosan nanoparticles, and subsequently increase the ocular retention time of the loaded drugs and/or the ocular penetration of the chitosan nanoparticles, decrease the initial burst, and increase the ocular bioavailability of the drugs [95, 99]. Table 8 summarizes various drug-loaded chitosan-based hybrid nanoparticles and their characteristics.

Polyionic complex nanoparticles made of hybrid chitosan and sodium alginate could continuously release antibiotic gatifloxacin [95] and betamethasone sodium phosphate [96] for 24–72 h *in vitro* and generated smaller initial burst for gatifloxacin than the chitosan or alginate alone [95]. An *ex vivo* permeability study showed that chitosan-alginate nanoparticles could penetrate through the rabbit sclera. *In vivo* studies of these nanoparticles in rabbits showed sustained release of betamethasone sodium phosphate for 12–24 h depending on the formulations when compared with the drug solution alone which disappeared after 2 h [96]. Zhu *et al.* [121] synthesized thiolated chitosan and evaluated the potential of thiolated chitosan-sodium alginate nanoparticles for ocular delivery. Results from their *in vitro* cytotoxicity study showed that the thiolated chitosan-sodium alginate nanoparticles were not cytotoxic to human corneal epithelial cells (HCE). Compared with chitosan-sodium alginate nanoparticles, thiolated chitosan-sodium alginate nanoparticles were smaller, more stable and adhesive, showed greater intracellular uptake by HCE cells, and delivered more FITC (model drug) into corneas [121].

Alonso and associates investigated the effectiveness of hyaluronic acid (HA) modified chitosan (chitosan-HA) nanoparticles for delivering genes to the cornea and conjunctiva [97, 122]. The zeta potential values of the chitosan-HA nanoparticles varied from positive to negative with increasing HA content. The transfection results demonstrated that the chitosan-HA nanoparticles were able to render high transfection levels to immortalized human corneal epithelial and normal human conjunctival cells (up to 15% of cells transfected), without affecting cell viability. After topical instillation into rabbit eyes, the chitosan-HA nanoparticles were internalized into the conjunctiva more than the cornea. Confocal images revealed that the internalization of chitosan-HA nanoparticles was due to fluid endocytosis, and the process was mediated by hyaluronan receptor CD44 (Figure 7). The nanoparticles were evenly distributed in corneal epithelial cells, but unevenly in conjunctival cells with more intense localization in the apical and basolateral regions. Vyas and associates [103] evaluated the effectiveness of these chitosan-HA nanoparticles for delivering timolol maleate and dorzolamide hydrochloride to the eye for the management of

glaucoma. As expected, the addition of HA in the nanoparticles showed increased *ex vivo* mucoadhesion and transcorneal permeation of the drug through excised goat cornea when compared with chitosan nanoparticles alone. Both chitosan-HA and chitosan nanoparticles showed *in vitro* burst release. Most of the loaded timolol maleate was released out of the chitosan/chitosan-HA nanoparticles as timolol was molecularly dispersed in the nanoparticles, whereas only 20% of the loaded dorzolamide hydrochloride was released due to its crystalline form. The timolol/dorzolamide hydrochloride loaded chitosan-HA nanoparticles did not cause any apparent ocular irritation to albino rabbits. They reduced intraocular pressure significantly more in the fellow eyes but less in the contralateral eyes of albino rabbits after 24 h of instillation [103]. The chitosan-HA nanoparticles caused higher intraocular pressure (IOP) lowering and less systemic absorption of the two drugs timolol and dorzolamide hydrochloride than the chitosan nanoparticles and the drug solutions after the instillation [103].

Yuan X *et al.* [118] synthesized amphiphilic conjugates of hydrophilic chitosan and hydrophobic cholesterol (CH) and obtained CH modified chitosan (chitosan-CH) nanoparticles through self-aggregation, and evaluated their potential for drug delivery to the ocular surface. The formed nanoparticles had an average particle size of about 200 nm and an average zeta potential of about 44 mV. They showed good dispersion over the entire precorneal area of rabbits following topical administration. While some of the chitosan-CH nanoparticle suspension was observed to be drained into lacrimal sac through the lacrimal duct, 71.4% of the chitosan-CH nanoparticles were well retained at the precorneal area at 112 min post-administration due to the bioadhesion ability of chitosan. Subsequently, the chitosan-CH nanoparticles retained at the cornea and conjunctiva, but would hardly permeate into the iris/ciliary body and reach to the posterior segment of the eye due to the tight barrier of the cornea. Hydrophobic drug CsA was entrapped in these nanoparticles using a simple dialysis method. In comparison with the chitosan nanoparticles alone, chitosan-CH nanoparticles enhanced both the CsA loading content and loading efficiency of chitosan nanoparticles (9% and 73%, vs. 6.2% and 41.8%, respectively). The chitosan-CH nanoparticles showed sustained release of CsA for over 48 h compared with the chitosan nanoparticles (24 h), and less initial burst than the chitosan nanoparticles alone (60% released in 4 h vs. 62% released in 15 min) as shown in Figure 8 [118, 123]. The reason for the less initial burst was probably due to the hydrophobic nature of the cholesterol component. This group also showed that further incorporation of hydrophobic poly(lactic acid) into the chitosan-CH nanoparticles could significantly improve the loading efficiency of rapamycin by ~4 fold and slow rapamycin release by reducing the percentage of rapamycin released at 12 h from 85% to 50% [124]. Furthermore, the chitosan-CH/PLA nanoparticles improved the immunosuppressive effect of rapamycin in corneal transplantation in New Zealand rabbits as effectively as and even slightly better than rapamycin suspension in terms of the medial survival time of the corneal allografts (27.2 ± 1.03 vs. 23.7 ± 3.20 days).

Hybrid nanoparticles made of chitosan and Carbopol® with 294 nm particle size were used to load pilocarpine [98]. The subsequent *in vitro* and *in vivo* studies demonstrated that these hybrid nanoparticles could slowly release pilocarpine for 24 h to decrease the pupil diameter of rabbits (miosis effect) more effectively than pilocarpine in liposome, gel and eye drop

formulations. Hybrid nanoparticles made of chitosan and dextran sulfate with around 400 nm particle size and around 40 mV zeta potential were stable in the presence of lysozyme for at least 4 h and showed at least 60 min prolonged adherence to the *ex vivo* porcine corneal surface [117]. After topical instillation of mucoadhesive chitosan-dextran sulfate nanoparticles (~400 nm size/+48 mV zeta potential; FITC labelled), the nanoparticles showed prolonged retention on porcine ocular surface for more than 4 h, and could be endocytosed into the *ex vivo* porcine corneal epithelial cells via clathrin-dependent pathway (Figure 9) [125]. Furthermore, after 6 h of topical administration, theses chitosan-dextran sulfate nanoparticles were observed to not be able to penetrate through corneal epithelium into stroma (Figure 10) [125]. Beta-cyclodextrin and its derivatives have a unique property of forming inclusion complexes with hydrophobic drugs to increase these drugs' water solubility due to their hydrophobic central cavity made of seven glucose units [126, 127]. In ocular application, sulfobutyl ether β-cyclodextrin was used to make hybrid nanoparticles with chitosan to form inclusion complexes with econazole nitrate to increase the water solubility of econazole nitrate. The resulting econazole nitrate-containing complexes showed improved bioavailability and prolonged 8 hours' steady antifungal effect while econazole nitrate solution could provide an antifungal effect for only 3 hours which also dramatically decreased with time [99].

Hybrid nanoparticles made of chitosan and lecithin had a particle size of ~100 nm and zeta potential value of around 27 to 43 mV, and were used for controlled release of natamycin [100], amphotericin B [101], and celecoxib [102]. *In vitro* release of natamycin showed a biphasic release profile with an initial burst release ~41.23% in 2 h followed by a slow release ~64.22% in 7 h [100]. However, *in vitro* release of celecoxib showed 24 h sustained release without initial burst [102]. *In vivo* ocular pharmacokinetics study of natamycin in New Zealand rabbits showed that the hybrid chitosan-lecithin nanoparticles increased the $AUC_{(0\text{-}\infty)}$ of natamycin 1.47-fold and decreased the clearance of natamycin 7.4-fold compared with the marketed natamycin suspension [100, 101]. *In vivo* ocular pharmacokinetics study of amphotericin B in albino rabbits showed that the hybrid chitosan-lecithin nanoparticles significantly improved the bioavailability (~2.04-fold) and precorneal residence time (~3.36-fold) of amphotericin B compared with amphotericin B solution (Fungizone®) available in the market [101].

3.2.3. Nanoparticles made of other polysaccharides—Other polysaccharides such as pectin, gum cordia, carboxymethyl tamarind kernel polysaccharide, galactomannan polysaccharide, and hyaluronic acid have also been used to make nanoparticles for ocular drug delivery [104–107, 128]. In an *ex vivo* study, pectin nanoparticles were found to significantly increase the permeability of timolol maleate across the excised goat cornea 200% more than the conventional timolol maleate ophthalmic solution [104]. On the other hand, gum cordia and carboxymethyl tamarind kernel polysaccharide nanoparticles were found to not enhance the permeation of loaded drugs fluconazole and tropicamide across the goat cornea as compared with the respective drug ophthalmic solutions [105, 107]. The authors speculated that even though these two types of nanoparticles did not increase the drug *ex vivo* corneal permeability, they might be able to provide sustained drug delivery *in vivo* due to their prolonged residence time in the *cul-de-sac.* Horvat *et al.* demonstrated that

the gel formulations made of nanosized crosslinked sodium hyaluronate, prepared by crosslinking via the carboxyl groups of the HA chains with a diamine using a carbodiimide technique, exhibited the highest capability of mucoadhesion compared with the gels made of linear HA derivatives (linear sodium hyaluronate, zinc hyaluronate). The explanations were that the interchain crosslinking structure and nanosize dimensions might enable the crosslinked sodium hyaluronate to interpenetrate easily and form secondary bonds with the mucin. Despite their differences in chemical and physical structures, all the above three types of nanoparticles showed an initial burst release of model drug sodium diclofenac at ~35–55% in 1 h, and released up to ~60–88% at 6 h [106]. Table 9 summarizes various drug-loaded nanoparticles made of other polysaccharides and their characteristics.

3.3. Polyester nanoparticles

Polyesters such as poly(lactide) (PLA), poly(lactide-*co*-glycoside) (PLGA) and poly(ε-caprolactone) (PCL) (Table 2) are synthetic polymers which are biodegradable with good biocompatibility. These polymers have been extensively used to fabricate implants, microparticles, and nanoparticles for controlled drug release and targeted drug delivery. In particular, nanoparticles fabricated from PLA, PLGA and PCL have been evaluated for drug delivery to the eye [129–135]. Drug or non-drug-loaded PLA, PLGA and PCL nanoparticles have been fabricated using solvent displacement (*i.e.* nanoprecipitation) [132–134, 136–138], and oil-in-water (o/w) emulsion solvent evaporation techniques [135, 139]. The resulting nanoparticles have particle sizes in the range of a hundred to a couple of hundred nanometers and bear negative zeta potentials. Hydrophobic drugs such as sparfloxacin, indomethacin, cyclosporine A, natamycin and flurbiprofen and hydrophilic drugs such as brimonidine and diclofenac sodium were encapsulated in these polyester nanoparticles. Below are detailed discussions about how PLA, PLGA and PCL and their derivative nanoparticles have been investigated to mask drug irritation to the eye, improve drug loading in the formulations, increase drug retention time in the precorneal area, and/or enhance drug penetration into/across the cornea or the conjunctiva-sclera barrier to reach the inner anterior segment [130, 132, 134, 137, 139–142].

Self-assembled polymeric micelles were synthesized from amphiphilic copolymers bearing a hydrophobic poly(2-methyl-2-carboxytrimethylene carbonate-*co*-*D,L*-lactide) backbone and a hydrophilic poly(ethylene glycol)-azide pendant chain [143]. GRGDS peptide was conjugated to the self-assembled micelles to target corneal epithelial cells through RGD receptors. *In vitro* evaluation of the binding affinity of these GRGDS-modified micelles to rabbit corneal epithelial cells showed that GRGDS-modified micelles could significantly inhibit the attachment of corneal epithelial cells to GRGDS-coated plates, indicating these micelles could be used for targeted drug delivery to the injured eye. PLGA nanoparticles prepared by nanoprecipitation were tested for *ex vivo* permeability across the cornea of New Zealand rabbits by Vega *et al.* [134]. The results showed that the PLGA nanoparticles considerably increased the penetration of flurbiprofen across the cornea by 4-fold over free flurbiprofen PBS (pH 7.4) solution and by about 2-fold over the commercial Ocuflur™ eye drops [134]. Corneal hydration study indicated that the corneas were not damaged during the 6 h *ex vivo* corneal permeability study. Similar drug penetration enhancement was observed on *ex vivo* goat corneas for sparfloxacin-loaded PLGA nanoparticles [137]. Further, *in vivo*

ocular retention study on New Zealand rabbits using scintigraphy studies revealed that radiolabeled sparfloxacin-loaded PLGA nanoparticles were cleared at a much slower rate and retained for a much longer duration at the corneal surface than commercial sparfloxacin eye drops. This was evidenced by the facts that after topical instillation radioactivity was detected in the systemic circulation (kidney and bladder) at 6 h with radiolabeled sparfloxacin eye drops but not with radiolabeled sparfloxacin-loaded PLGA nanoparticles; and radioactive counts at corneal surface within the first 0.5 h quickly decreased with radiolabeled sparfloxacin eye drops but remained nearly constant with radiolabeled sparfloxacin-loaded PLGA nanoparticles [137]. Since PLGA nanoparticles are hydrophobic and negatively charged at the surface, they are non-mucoadhesive in nature [137]. Therefore, the observed long retention for the nanoparticles would probably due to the particle size of the nanoparticles (180–190 nm) [137, 144]. In addition to the PLGA nanoparticle size effect, the poly(vinyl alcohol) stabilizer was also found to help to increase the nanoparticle retention time by increasing the viscosity of the nanoparticle dispersion due to the hydrophilic nature of the poly(vinyl alcohol) polymer [137, 140]. Diclofenac sodium-loaded PLGA and poly (lactide-co-glycolide-leucine) polymeric nanoparticle suspensions with size ~125–190 nm, and zeta potential ~-25 mV showed a biphasic release pattern of the drug: one initial fast release (~50% in first 2 h) followed by a second slow-release phase for up to 14 h, and the particles were observed to be free of any irritant effect on cornea, iris, and conjunctiva for 24 h after topical application in rabbit eyes [142]. PCL nanocapsules have been studied for topical ocular drug delivery by Alonso and co-workers [136, 140, 145]. These PCL nanocapsules were made from PCL, lecithin and Miglitol 840 using solvent displacement technique. The fluorescent dye loaded PCL nanocapsules with a mean size of 252 nm have enhanced the penetration of rhodamine 6G through the corneal epithelial cells of New Zealand rabbits by endocytosis *ex vivo*, and were selectively internalized into the corneal epithelium cells (vs. the conjunctival cells) after topical instillation to the *cul-de-sac* of fully awake New Zealand rabbits [140]. PCL nanocapsules loaded with indomethacin had an average particle size of 238 nm and a zeta potential of −39 mV and were well tolerated by New Zealand rabbits, and significantly increased the indomethacin concentrations in the cornea (~3.2-fold increase in AUC) and aqueous humor (~4.3-fold increase in AUC) than commercial eye drop Indocollyre after topical instillation to the *cul-de-sac* of the right eyes in New Zealand rabbits [136]. After PCL nanoparticles had been functionalized with poly-*D*-glucosamine, the obtained nanoparticles prolonged the release of natamycin for up to 8 h *in vitro,* and significantly increased relative bioavailability (~6.03–6.39-fold) compared to the commercial natamycin suspension (Natamet®) [138]. Ibrahim *et al.* loaded brimonidine into PLA (molecular weight 152 kDa), PLGA 75:25 (molecular weight 66–107 kDa) and PCL (molecular weight 14 kDa) nanoparticles which had 117~131 nm mean particle size, −18~ −28.11 mV zeta potential (Figure 11) [135]. The nanoparticles could encapsulate 68–78% of brimonidine, and the encapsulation efficiency increased in the order of PLGA<PLA<PCL with increasing the hydrophobicity of the polymers and the consequent solid-state solubility of the hydrophobic brimonidine freebase in the polymers. The *in vitro* release rates of brimonidine from the nanoparticles increased in the order of PCL>PLGA>PLA due to the decrease of the molecular weights of the polymers used in the nanoparticle preparations. Ibrahim *et al.* further embedded the brimonidine-loaded nanoparticles into methyl cellulose-based gels and demonstrated that the resulting

brimonidine-nanoparticle-gel systems had significant better IOP lowering effect than brimonidine-containing Alphagan P® eye drops (effective duration 15.2–23.2 vs. 7–7.4 h) after instillation in BXD (boxed molecular dynamics) mice [135].

To increase the precorneal residence time and consequent ocular bioavailability of drug-loaded PLA, PLGA, and PCL polyester nanoparticles, mucoadhesive polymers chitosan [89, 131, 136, 146], cholesterol modified chitosan [124], Carbopol® [139] and hyaluronic acid (HA) [129, 147] were used to form a coating layer on the nanoparticles. Among these mucoadhesive polymers, chitosan is the most extensively exploited one. Chitosan coating was introduced into the polyester nanoparticles during the nanoparticle synthesis by using a chitosan-containing aqueous solution. This chitosan coating significantly shifted the zeta potential of the nanoparticles from negative (uncoated) to positive (coated) value [124, 131, 136, 146]. The positively charged nanoparticles can interact with the negatively charged mucus layer of the tear film and the negatively charged outer layer of the cornea to prolong the retention time of drugs on the corneal surface and increase the penetration of drugs across the cornea. *Ex vivo* permeability studies showed that by loading rhodamine 6G into chitosan-coated PCL nanoparticles or simply mixing rhodamine 6G with blank chitosan-coated PCL nanoparticles, the transport of rhodamine 6G across the rabbit cornea was significantly enhanced more (1.9–3.3-fold in 4 h) compared with uncoated PCL nanoparticles [131]. Similar observation was reported on the *ex vivo* study of the permeability of rhodamine 6G-loaded chitosan-coated PLGA nanoparticles across the goat cornea [146]. *In vitro* studies demonstrated that the chitosan coating on PCL nanoparticles did not modify indomethacin release kinetics from the PCL nanoparticles, but strongly helped to increase the indomethacin concentrations in the cornea and aqueous humor of rabbits more than uncoated PCL nanoparticles and commercial eye drop Indocollyre after topical instillation [136]. However, when PCL nanoparticles were coated with neutral PEG, the transport of loaded rhodamine 6G across the *ex vivo* rabbit cornea was not increased [131]; when PCL nanoparticles were coated with positively charged PLL, the ocular bioavailability of indomethacin in rabbit cornea and aqueous humor was not increased either [136], in comparison with uncoated PCL nanoparticles. The possible explanation for the enhanced corneal transport and ocular bioavailability by the chitosan coating but not the PLL and PEG coatings might be that the chitosan significantly increased the zeta potential of the PCL nanoparticles from −39.9 to 37.1±1.8 mV [136] more than the PLL and PEG (27.9±3.5 and −40.2 ± 1.7 mV [131, 136], respectively), and had strong mucoadhesive nature. A recent study showed that cholesterol-modified chitosan/PLA nanoparticles mainly remained on the cornea and conjunctiva too for about 150 min after topical instillation, which is about 2–6 times higher than the drug suspension [124]. Researchers also used negatively charged Carbopol® (crosslinked polyacrylic acid) to coat PLGA nanoparticles, and obtained slightly larger (393 vs. 219 nm) and more negatively charged nanoparticles compared with uncoated PLGA nanoparticles [139]. *In vitro* uptake studies showed that the Carbopol® coated PLGA nanoparticles were taken up by L929 mouse fibroblast cells more than the corresponding uncoated PLGA nanoparticles (40% vs. 33.2% after 6 h exposure). This increased cellular uptake might be attributed to the adhesive properties gained by the nanoparticle through the Carbopol® coating. The bioadhesive properties imparted by Carbopol® were probably due to the entanglement between Carbopol® and mucin layer

covering the corneal epithelium and the hydrogen bonding between the carboxylic groups of Carbopol® and the glycoproteins of the mucin layer [139]. Barbault-Foucher *et al.* coated PCL nanospheres with hyaluronic acid (HA) and investigated the effect of cationic surfactants stearylamine and benzalkonium chloride (BKC) on the coating [129]. Their results showed that both of the cationic surfactants enhanced the adhesion of HA on PCL nanosphere surface, and BKC had a higher effect than stearylamine (41.6 vs. 26.4 mg HA/mg PCL). Yenice *et al.* further studied the effects of the PCL/BKC nanospheres with and without HA coating on CsA corneal delivery in healthy rabbits [147]. Both PCL/BKC and HA coated PCL/BKC nanospheres increased CsA corneal delivery 10–15 times more than CsA solution in castor oil. Furthermore, the HA coated PCL/BKC nanospheres delivered much more CsA in the cornea than the uncoated PCL/BKC nanospheres (11.4–23.0 vs. 5.9–15.5 ng/mg tissue) 4 h post-topical administration. Phenylboronic acid that can form a complex with *cis*-diol groups of sugar units, such as sialic acid, ample in mucin at physiological pH [148, 149], [added comma] was used to functionalize the surface of poly(*D,L*-lactide)-*b*-dextran nanoparticles to increase the precorneal residence time of the nanoparticles [150]. The surface functionalization with phenylboronic acid led to change in the size and zeta potential of the nanoparticles from 45.8 to 28.6 nm and −2.63 to −31.3 mV, respectively; and high mucin binding of the nanoparticles: 1.18 ± 0.02 mg per mg nanoparticles, which was much greater that the values attained with other types of mucoadhesive nanoparticles such as chitosan-based nanoparticles (~ 0.25 mg per mg nanoparticles) and thiolated nanoparticles (~ 0.13 mg per mg nanoparticles). Slit-lamp examination and histopathology observations in rabbits demonstrated that phenylboronic acid surface functionalized poly(*D,L*-lactide)-*b*-dextran nanoparticles (~35.6 nm) loaded with and without CsA (12% loading) did not show any detectable irritation or inflammatory reactions after weekly administration for up to 12 weeks. The nanoparticles sustained release CsA for 5 days *in vitro*, and the released CsA showed efficacy in treating experimental dry eye-induced mice *in vivo*: once a week administration of CsA loaded nanoparticles achieved both the elimination of inflammatory infiltrates and recovery of the ocular surface, whereas thrice a day administration of commercial product Restasis® (Cyclosporine Ophthalmic Emulsion, 0.05%) only showed clearance of the inflammatory processes [150]. Table 10 summarizes various drug-loaded polyester nanoparticles and their characteristics.

3.4. EUDRAGIT® polymer nanoparticles

The hypothesis that the positive charges bearded by nanoparticles can facilitate an effective adhesion to the ocular surface stimulated the development of nanoparticles made of cationic EUDRAGIT® for topical ocular drug delivery [151–160]. EUDRAGIT® is made of poly(ethyl acrylate-methyl methacrylate-chlorotrimethyl-aminoethyl methacrylate) (Table 2) containing quaternary groups between 4.5–6.8% and 8.8–12% for RS and RL grades, respectively. Nanoparticles made of EUDRAGIT® were fabricated by nanoprecipitation [155], spontaneous emulsification [156], and quasi-emulsion solvent diffusion techniques [151, 152]. These EUDRAGIT® nanoparticles demonstrated good ocular tolerance in rabbit eyes, and did not cause any inflammation in the ocular tissues [151, 155, 161, 162]. Pignatello and his group encapsulated ibuprofen and flurbiprofen into EUDRAGIT® RS nanoparticles and tested their effect in preventing miosis caused by surgical traumas [151, 152] and ocular inflammation [151]. Compared with the corresponding eye drops,

EUDRAGIT® RS nanoparticles enhanced the presence of the loaded drugs (ibuprofen and flurbiprofen) in the aqueous humor of rabbit eyes [151, 152]. This increase of drug concentration and prolonged drug persistence in the aqueous humor provided a high capacity to inhibit miosis and inflammation in the anterior segment of the rabbit eye. These results were attributed to the enhanced drug retention at the eye surface due to the encapsulation of the drugs in the nanoparticles, and a progressive and continuous release of the incorporated drugs from the nanoparticles adhered on the cornea surface [151, 152]. Amikacin loaded EUDRAGIT® RS 100/RL 100 nanoparticles were prepared and assessed for *in vitro*, *in vivo* and pharmacokinetic performances [163]. They had a mean particle size 122–242 nm, and zeta potential 29–46 mV depending on the proportion between RS 100 and RL 10. *In vivo* ocular retention study by scanning electron microscope revealed that the EUDRAGIT® nanoparticles exhibited corneal adherence for prolonged 6 h after instillation in healthy male albino rabbits. The EUDRAGIT® nanoparticles significantly increased the C_{max} ~1.733 times and $AUC_{0–12\ h}$ ~2.12 times of amikacin in aqueous humor more than the commercial ophthalmic solution of amikacin sulfate [163]. Hybrid EUDRAGIT® nanoparticles were prepared using o/w emulsification-solvent evaporation method from mixtures of PLGA and EUDRAGIT® RL with different weight ratios (75:25, 50:50 and 25:75) [139]. The particle size of the obtained hybrid nanoparticles slightly increased with the increase of the PLGA content, and the nanoparticles' zeta potential was positive and was not affected by the EUDRAGIT® RL content. An *in vitro* uptake study showed that the hybrid EUDRAGIT® nanoparticles (75:25) was taken up by L929 cells much more than Carbopol® coated PLGA and uncoated PLGA nanoparticles (50% vs. 40% and 33.2%, respectively after 6 h) (Figure 13). An *in vivo* pharmacokinetic study revealed that the hybrid EUDRAGIT® nanoparticles (75:25) gave the highest $AUC_{0–24\ h}$ (972.59 ng·h·g^{-1}) and C_{max} (366.30 ng·g^{-1}) of cyclosporine A in the rabbit tear, compared with the Carbopol® coated PLGA ($AUC_{0–24\ h}$ 776.57 ng·h·g^{-1} and C_{max} 211.08 ng·g^{-1}) and uncoated PLGA ($AUC_{0–24\ h}$ 490.42 ng·h·g^{-1} and C_{max} 126.12 ng·g^{-1}) nanoparticles. The higher bioavailability in case of Hybrid EUDRAGIT® nanoparticles could be the result of ionic interaction between the negatively charged mucin layer at the eye surface and the cationic EUDRAGIT® RL nanoparticles where these interactions offer the possibilities of prolonging the residence time of the formulation in the eye. Hyaluronic acid (HA) was used to coat EUDRAGIT® nanoparticles aiming for prolonged ocular delivery of gatifloxacin/prednisolone biotherapy [156]. These HA coated EUDRAGIT® nanoparticles were prepared by either coating preformed EUDRAGIT® nanoparticles with HA or applying the HA coating during the synthesis of EUDRAGIT® nanoparticles via a spontaneous emulsification method. The latter process produced much larger HA coated nanoparticle (678.00 nm) compared with the process of coating preformed nanoparticles (460.30 nm). However, the both types of HA coated nanoparticles had similar zeta potential value of about −45 to −47 mV. The HA-coated nanoparticles provided significantly increased and prolonged drug concentrations in the cornea (C_{max} 5.2-fold, $AUC_{0–6\ h}$ 7.9-fold) and aqueous humor (C_{max} 1.8-fold, $AUC_{0–6\ h}$ 1.8-fold) of rabbit eyes *in vivo* compared with the commercial eye drops. However, no comparison between the coated nanoparticles and uncoated nanoparticles was reported in the paper [156]. Currently, in the literature, there is no report about the limitation of EUDRAGIT® or EUDRAGIT® nanoparticles. Based on general knowledge, one obvious limitation of EUDRAGIT® is that the polymer poly(ethyl acrylate-methyl methacrylate-

chlorotrimethyl-aminoethyl methacrylate) for making EUDRAGIT® nanoparticles is not biodegradable. Further studies about the efficacy and side effects of EUDRAGIT® nanoparticles are needed in the future. Table 11 summarizes various drug-loaded EUDRAGIT® based nanoparticles and their characteristics.

3.5. Lipid nanoparticles

Lipid nanoparticles including solid lipid nanoparticles and nanostructured lipid carriers emerged in the 90s as an alternative drug delivery system to liposomes and polymeric nanoparticles [164, 165]. Lipid nanoparticles have an advantage over liposomes in terms of low cost for large scale production and less storage and drug leakage issues [166, 167]. They have advantages over biodegradable polymeric nanoparticles in terms of less significant toxicity and acidity problems [166, 167]. Lipid nanoparticles have been extensively investigated in the academic and pharmaceutical industry as carriers for dermal, mucosal, oral, intravenous/parenteral, pulmonary and ocular delivery of small molecule drugs, peptides/proteins and genes [168]. Lipid nanoparticles usually have a size in the range of 50~1000 nm and remain in a solid state at body temperature. They are synthesized from various lipids such as lipid acid, monoglycerides, diglycerides, triglycerides, glyceride mixtures and waxes using high-pressure homogenization, microemulsion, solvent emulsion-evaporation/diffusion, double emulsion, high-speed stirring and/or ultrasonication techniques [168–171].

The application of solid lipid nanoparticles (SLN) for ocular drug delivery traced back to 2002 when Cavalli *et al.* reported their study on tobramycin-loaded solid lipid nanoparticles [172]. Cavalli *et al.* synthesized tobramycin-loaded SLN from stearic acid, Epikuron 200, sodium taurocholate and water using a warm o/w microemulsion method. They obtained 70~80 nm SLN that were well tolerated by rabbit eyes and did not cause any ocular irritation. An *in vivo* ocular pharmacokinetics study showed that the SLN could deliver tobramycin in the aqueous humor of rabbit eyes, and increased tobramycin C_{max} 1.5-fold, T_{max} 8-fold and $AUC_{0–6\ h}$ 4-fold more than an equal dose of commercial tobramycin eye drops (Tobral®) after topical instillation. This enhanced tobramycin bioavailability by SLN might be attributed to the entrapment and retaining of the SLN in the mucin layer covering the corneal epithelium, and the Epikuron 200 component in the SLN which helped to enhance the corneal penetration of tobramycin. Caramella and her group developed a CsA-loaded SLN using Compritol 888 ATO (glyceryl behenate) as a solid lipid, and Poloxamer 188 and Tween 80 as surfactants via a high shear homogenization and ultrasound method [173, 174]. They obtained optimized Compritol-SLN with a particle size of 225 nm with and without CsA and a zeta potential value of −16.9 mV and −23.6 mV with and without CsA, respectively. The CsA loading efficiency could reach as high as 95.6%. They found that Compritol-SLN nanoparticles sterilized at 110 °C showed no cytotoxicity to corneal epithelial cells (RCE) *in vitro* and did not induce ocular irritation in rabbit determined by the Draize test. However, Compritol-SLN sterilized at 120 °C leaked out surfactant Tween 80 and induced cytotoxicity to REC cells *in vitro*. Compared with CsA suspension, the Compritol-SLN nanoparticles significantly enhanced the *ex vivo* penetration of CsA through the porcine cornea 10-fold more. Further, an *in vivo* study demonstrated that CsA could penetrate into the aqueous humor at a therapeutic level two hours after topical instillation of

CsA-loaded SLN into rabbit eyes. To improve SLN's interaction with and internalization in corneal cells, Caramella and her group added cationic chitosan during the SLN synthesis [175]. Başaran *et al.* also [176] reported that octadecylamine-modified cationic SLN with size 248±0.33 nm and zeta potential - 50.30±0.78 mV could continuously release CsA in the aqueous humor and vitreous humor of sheep eye for more than 48 h after topical instillation. Leonardi *et al.* fabricated melatonin-encapsulated cationic SLN using Softisan 100 as the main lipid matrix, didecyldimethylammonium bromide as a cationic lipid, stearic acid or palmitic acid as a lipid modifier, tween 80 as a surfactant, and lipid didecyldimethylammonium bromide as a cationic surface lipid [177]. The obtained nanoparticles had a size range of 150~300 nm and polydispersity index (PDI) < 0.35 with positive surface charge, and caused significant IOP lowering in albino rabbits for about 24 h. Using compritol and palmitic acid as lipid carriers, Poloxamer 188 and soya lecithin as surfactants, and sodium taurocholate as a co-surfactant, Kumar *et. al* prepared SLN of size range 139 – 334 nm and zeta potential >-30 mV for the ocular delivery of voriconazole [178]. The SLN exhibited sustained release of voriconazole (~60–80% in 12 h vs. ~100% in 6 h for drug suspension). Histopathology (Figure 14) studies of excised goat cornea and HET-CAM studies (Figure 15) treated with the SLN, irritant (0.1 N) and control (0.9% NaCl) demonstrated that the SLN were non-irritant to the cornea [178]. *In vivo* pharmacokinetics study of the SLN in rabbits revealed that the SLN significantly increased the bioavailability of voriconazole with C_{max} 1.4-fold, $AUC_{0–12\ h}$ 2-fold, and mean residence time (*MRT*) 1.2-fold increases in comparison to the plain drug suspension [178]. Sharma *et. al.* prepared GMS based SLN and found that the ^{99m}Tc labeled SLN greatly prolonged celecoxib retention on the rabbit ocular surface than the drug aqueous suspension (20% vs. 75% drop in the intensity) by using gamma scintigraphy [179]. The GMS based SLN with size around 200 nm could significantly increase the permeation of celecoxib across the rabbit cornea compared to celecoxib aqueous suspension (permeation 8.21±0.67% vs. 4.61±0.71%) [179].

Nanostructured lipid carriers (NLC) appeared in the late 90s and early this century to overcome the limitations associated with solid lipid nanoparticles such as limited drug loading, the risk of gelation and drug leakage during storage [170]. In NLC, spatially incompatible liquid lipids were incorporated into the solid lipids to generate structure defects in the crystals for the accommodation of drugs [180, 181]. In 2008, Pan and his group first reported NLC for ocular drug delivery [182]. They fabricated NLC from Compritol ATO, Gelucire 44/14, stearylamine, Miglyol 812, Transcutol and/or Cremophor EL using a melted-ultrasonic method. They found that the obtained NLC with an average particle size of 69 nm and a zeta potential value of +28.9 mV were well tolerated by rabbit eyes, increased the *ex vivo* permeability of ibuprofen across the rabbit cornea 4.9-fold, and increased the *AUC*, C_{max}, and T_{max} of ibuprofen in the rabbit aqueous humor 3.99-, 3.25- and 3.2-fold, respectively, compared with conventional ibuprofen eye drops. The enhanced bioavailability in the aqueous humor might be attributed to the Gelucire 44/14 and Transcutol P components in the NLC which enhanced the permeability of ibuprofen across the cornea, and the stearylamine component in the NLC which prolonged the precorneal retention of ibuprofen. Following this pioneering report, more groups devoted effort to the development of NLC for ocular drug delivery including anterior eye segment drug delivery

[180, 183–190]. One attempt was to further improve the precorneal retention and/or transcorneal penetration of drugs by incorporating mucoadhesive polymers/ligands in NLC. Chitosan oligosaccharides [186], partially deacetylated water-soluble chitosans [188], and N-acetyl-L-cysteine functionalized chitosan (CS-NAC) [191] were used to coat preformed NLC fabricated from Compritol 888 ATO, Gelucire 44/14, and Miglyol 812 using melted-emulsification and ultrasonication techniques. The chitosan coating layer increased the sizes of the NLC ~21–40% and rendered positive zeta potential to the NLC. The chitosan-coated and uncoated NLC did not cause any irritation to rabbit corneas and conjunctivas. The chitosan coating increased the precorneal retention of drug flurbiprofen and the penetration of the drug across the *ex vivo* rabbit cornea, depending on the types and amounts of the chitosan coatings [186, 188]. Mucoadhesive thiolated PEG stearate was also incorporated into a NLC fabricated from Precifac ATO® 5 and Miglyol® 840 for ocular drug delivery [185, 189]. The thiolated PEG stearate did not change the NLC's size (around 60 nm) and zeta potential (−12 to −18 mV). CsA loaded NLC with and without thiolated PEG stearate could sustain release of CsA for more than 2 days *in vitro* and the thiolation of NLC could slightly decrease the *in vitro* drug release rate [189]. The thiolated NLC were non-irritant to rabbit eyes, increased the residence time of CsA on the rabbit cornea surface to 6 h, at least 1.5 times more than non-thiolated NLC, and delivered ~2–15 times more CsA to the cornea, conjunctiva, iris-ciliary body, aqueous humor and tear fluid than castor oil solution eye drops of CsA, NLC and PEG-NLC 24 h after instillation into the lower *cul-de-sac* of rabbit eyes. The CsA loaded thiolated PEG searate NLCs could deliver 934.96 ng/g of CsA to the iris-ciliary body of rabbit eyes with good immunomodulation effect. These results suggested a direct correlation between the thiol group composition and the mucoadhesive behavior of the NLC: the thiol group formed disulfide bonds with the cysteine-rich subdomains of mucus glycoproteins to enhance the mucoadhesion of the NLC [185, 192]. Liu *et al.* fabricated CS-NAC coated NLC that had particle size ~50 to 88 nm and zeta potential ~ −20 to +22 mV [191]. They found that the *ex vivo* and *in vivo* transcorneal permeability of curcumin was increased in the order of solution eye drops < NLC < chitosan-coated NLC < CS-NAC-coated NLC with C_{max}, $AUC_{0-\infty}$ and $MRT_{0-\infty}$ increasing 3.88-, 5.97- and 1.27-fold, 5.28-, 12.25- and 2.19-fold, and 8.88-, 29.88- and 2.9-fold, respectively, in comparison to the drug eye drops [191]. They explained that the reason for the NLC to increase the transcorneal permeability of curcumin more than the drug solution was attributed to the presence of the lipid matrix in the NLC which could adhere to corneal epithelial cells to increase the residence time of curcumin on the cornea surface. The reason for the chitosan-coated NLC to increase the transcorneal permeability of curcumin more than the NLC alone was attributed to the electrostatic interaction of the positively charged chitosan-coated NLC with the cornea surface which could lead to temporary loosening of tight junctions between corneal epithelial cells to increase the penetration of the drug. The highest permeability observed with the CS-NAC-coated NLC was because of the potential opening of tight junctions between corneal epithelial cells through inhibition of protein tyrosine phosphatase by thiolation and the mucoadhesive nature of the N-acetyl-L-cysteine coated on the NLC. Amphipathic octadecyl-quaternized carboxymethyl chitosan was also used as a cationic material to be coated on NLC to improve the transcorneal permeability and ocular bioavailability of curcumin [193]. The obtained nanoparticles had particle size 158 nm and zeta potential +36.5 mV, increased the *in vivo* corneal permeability of curcumin in rabbits

more than the NLC alone with C_{max}, $AUC_{0-\infty}$ and $MRT_{0-\infty}$ 1.3-,2.4- and 2.3-fold vs. 1.2-, 1.5- and 1.8-fold increase, respectively, in comparison to the drug eye drops. The above results indicated that N-acetyl-L-cysteine functionalized chitosan was more promising than amphipathic octadecyl-quaternized carboxymethyl chitosan to be used as a coating for NLC for improving the ocular bioavailability of curcumin [191, 193].

Ban *et al.* modified dexamethasone-loaded lipid nanoparticles made of soy/lecithin by chitosan coating and observed that the modification resulted in a 1.5-fold increase in apparent permeability coefficient of the drug dexamethasone [194]. The chitosan-modified lipid nanoparticles increased the dexamethasone ocular bioavailability more than the unmodified ones in rabbits with C_{max}, T_{max} and $AUC_{0-24\ h}$ increasing 2.37-, 12 and 4.69-fold vs. 1.17-, 10 and 2.12-fold, respectively in comparison to dexamethasone eye drops [194]. Liu *et al.* evaluated liquid crystalline nanoparticles (LCNP) for the delivery of tetrandrine using components including glyceryl monoolein, poloxamer 407, water, Gelucire 44/14 and amphipathic octadecyl-quaternized carboxymethyl chitosan [195]. *In vitro* corneal permeation test demonstrated that LCNP enhanced the apparent permeability coefficient of the drug by 2.03-fold compared to the drug solution. LCNP showed prolonged ocular residence time *in vivo*, and increased the ocular bioavailability of tetrandrine in rabbits by 2.85-fold compared to the drug solution [195]. Table 12 summarizes various drug-loaded lipid-based nanoparticles and their characteristics.

3.6. Dendrimers

Dendrimers are nanoscale, highly branched, and reactive three-dimensional macromolecules with a high degree of molecular uniformity, narrow molecular weight distribution, specific size, and intriguing structural properties such as internal voids and cavities and a highly functional terminal surface formed by amino, carboxyl and/or hydroxyl groups [196, 197]. Since Tomalia *et al.* first synthesized polyamidoamine (PAMAM) dendrimers in the mid 1980's [198], dendrimers have been extensively studied for drug and gene delivery and diagnosis because drugs, genes, diagnostic agents, and targeting moieties can be encapsulated in the central core or physicochemically conjugated to the terminal surface of the dendrimers [199–202]. However, dendrimers have been explored for ocular drug delivery in only recent ten years [41, 43] since the first report by Vandamme *et al.* in 2005 [203]. Hydrophilic drugs pilocarpine nitrate [203], carteolol [204], timolol maleate [205, 206] gatifloxacin [207] and hydrophobic drugs tropicamide [203], acetazolamide [208], and brimonidine [205, 206] have been loaded into dendrimers for ocular drug delivery. In Vandamme *et al.*'s study, tropicamide and pilocarpine nitrate-loaded PAMAM dendrimers were evaluated for drug residence time on the cornea, and miotic and mydriatic activities, after instillation in the eyes of New Zealand albino rabbits [203]. The results showed that the dendrimer aqueous solutions had comparable pH, osmotic pressure, viscosity, surface tension, and refractive index as Carbopol 980 NF (0.2% w/v) eye drops with no disturbance in the visual acuity. The mean corneal residence times (~4–5 h) of the aqueous solutions of PAMAM dendrimers with generations G1.5(COOH), G2(OH) and G4(OH) were comparable to that of the Carbopol 980 NF (0.2% w/v) eye drops, while that of the dendrimer G2(NH2) was significantly shorter. The PAMAM dendrimers with carboxyl or hydroxyl terminal surface groups increased the miotic effect of pilocarpine nitrate with

1.39–1.64-fold increase in *AUC* and the mydriatic activity of tropicamide with 1.13–1.34-fold increase in AUC in comparison with 1% drug in phosphate buffer [203]. Spataro *et al.* developed phosphorus-containing dendrimers that had one quaternary ammonium salt as core and carboxylic acid as terminal groups for delivering carteolol to treat glaucoma [204]. They loaded carteolol into the dendrimers through ion pair interaction between the amino group of the neutral carteolol and the carboxyl group of the dendrimers. The water solubility of the obtained catanionic (saline) species decreased with increasing the generation of the dendrimers (G0-soluble; G1, G2-poorly soluble). The dendrimers were not irritant to the eyes of albino rabbits for at least 8 h after installation. Upon instillation, G0 dendrimers did not improve concentration; however, G2 dendrimers caused a 2.5 times increase in the concentration of carteolol in the aqueous humor in comparison with the drug suspension alone [204]. Durairaj *et al.* reported the development of dendritic polyguanidilyated translocators (DPT) as an ocular drug delivery vehicle to enhance the solubility and permeability of gatifloxacin [207]. The DPT nanoparticles that they synthesized had a size of around 346 nm and increased the water solubility of gatifloxacin by 4-fold. The nanoparticles enhanced the *ex vivo* permeability of gatifloxacin across the bovine sclera-choroid-RPE by 40%, and increased the *in vitro* killing effect of gatifloxacin to methicillin-resistant *Staphylococcus aureus* by a factor of 3. In comparison with the drug solution alone, the nanoparticles increased the bioavailability of gatifloxacin in the cornea and conjunctiva of New Zealand white rabbits by ~2- and ~13-fold, respectively; and prolonged the retention time of gatifloxacin in the rabbit aqueous humor from 2 to 24 h [207]. Mishra V *et al.* investigated the effectiveness of acetazolamide-loaded G5.0 poly(propylene imine) dendrimers in lowering the intraocular pressure in normotensive adult male New Zealand albino rabbits [208]. The dendrimers increased the corneal residence time of acetazolamide by ~5–7-fold depending on the dendrimer concentration, and prolonged the IOP-lowering effect of acetazolamide from 2 h to ~4 h in comparison with acetazolamide solution alone. Table 13 summarizes various drug-loaded dendrimer nanoparticles and their characteristics.

3.7. Nanoparticle-based composite systems

The above discussed nanoparticle systems have proven promising in terms of increasing the drug residence time, improving the ocular penetration of drugs and enhancing the ocular bioavailability of the drugs. However, as nanoparticles have small size and drug release duration is proportional to the particle size [209], to further improve the ocular therapeutic duration and bioavailability of drugs released from nanoparticles, nanoparticles are embedded in a matrix such as hydrogels or hydrogel contact lenses to form a composite drug delivery system [210–213]. In this composite system, a drug needs first to diffuse through the nanoparticle, reach the hydrogel/contact lens matrix and then diffuse through the hydrogel matrix to reach the target [214]. Therefore, the drug release duration of the composition system is longer than the nanoparticles alone and also the hydrogel/contact lens matrix alone [214, 215]. In addition, the composite system can also improve the biocompatibility of the nanoparticle carriers by hiding them within the hydrogel or contact lens matrix, to prevent the nanoparticles from migrating away from their intended target site *in vivo*, and to minimize the drug metabolism from the enzymes present in the tear/corneal surface [216, 217].

Several types of nanoparticles such as liposomes or lipid-based nanoparticles [210, 213, 218–220], micelles [221, 222], polymeric and metal nanoparticles [211, 214, 217] have been loaded in hydrogels/contact lenses to design extended-release composite systems with varying degree of success. Besides contact lenses, other composite systems including implants, fibers or *in situ* gels were also used to load nanoparticles [218, 223]. Drugs including lidocaine [210], ciprofloxacin [219], levofloxacin [220], CsA [221, 222], timolol [213, 217], silver [224], meloxicam [225], dexamethasone [226–228], loteprednol etabonate [214], hydrocortisone butyrate [223], and resina draconis [218] have been loaded into the nanoparticles to be delivered through the composite systems. Four different methods have been used to prepare nanoparticle-laden therapeutic contact lenses. The first most common method includes two steps: nanoparticles are first prepared and loaded with drugs at size 3–100 nm, and then dispersed in HEMA monomer solution to form nanoparticle embedded contact lens after polymerization of HEMA monomers is initiated [211, 214, 217]. The second method is attained by a single step process with surfactants included during the polymerization process to form micellar aggregates in the polymer hydrogel matrix to incorporate hydrophobic drugs [221, 222]. The third method is to load the nanoparticles into prefabricated lenses by soaking the lenses in a solution (e.g. ethanol) containing the nanoparticles [217]. The fourth method is to immobilize drug loaded nanoparticles on the surface of contact lenses [229, 230]. In this Section, we will review liposomes, lipid nanoparticles, micelles, polymeric and metal nanoparticle-based composite systems for drug delivery to the anterior segment.

3.7.1. Liposome/Lipid nanoparticle-based composite systems—The use of liposomes for incorporation of the drug within contact lens matrices was explored initially by Chauhan and his associates. Poly(2-hydroxyethyl methacrylate) (PHMEA) contact lenses encapsulated with lidocaine-loaded dimyristoyl phosphatidylcholine liposomes (size ~20 nm) showed a sustained *in vitro* release of lidocaine with 65% drug release in 7 days and an initial burst of about 15–30%, but had slightly lower transparency (80%) compared with the blank lenses (90%) [210]. Instead of being dispersed, Danion *et al.* immobilized levofloxacin loaded liposomes on the surface of commercial contact lenses (Hioxifilcon B) [220, 229]. The lenses that were surface immobilized with 2 layers of liposomes showed drug release for up to 30 h while those with 10 layers of liposomes released the drug for 120 h. However, the 10 layers of liposomes decreased oxygen and carbon dioxide permeability through the lens. The liposome-laden contact lenses were found to be non-toxic to human corneal epithelial cells, reconstructed human corneas and *ex vivo* rabbit corneas [220, 229]. Surface entrapment of liposomes did not affect the light transmission of the contact lenses compared with the control lenses without liposomes at the maximal photopic sensitive wavelength, and, in addition, the lens with the liposome layers afforded more eye protection in the ultraviolet spectrum, compared with the control lenses [230]. Jain *et al.* prepared drug-eluting contact lens by coating soft contact lenses with ciprofloxacin-loaded liposomes including unilamellar or multilamellar vesicles [219]. Multilamellar vesicles (mean diameter 338.32)-containing liposomes had a larger size, exhibited higher values of entrapment efficiencies (~1.5 to 3.9 times), and showed greater sustained action than unilamellar vesicle (mean diameter 120.42 nm)-containing liposomes. The liposomal formulations were found to be non-toxic and also to lower the toxicity of ciprofloxacin when evaluated by

lymphocyte toxicity assay and chick embryo inoculation. The contact lens coated with ciprofloxacin-entrapped liposomes showed much longer *in vitro* residence time than ciprofloxacin eye drops (24 h vs. 2–5 min) [219]. Yu *et al.* prepared a composite system wherein timolol maleate-containing liposomes (136±18 nm) were dispersed into deacetylated gellan gum-based ion sensitive *in situ* gels [213]. The composite system showed more sustained *in vitro* timolol maleate release than liposome nanoparticles alone (24 vs. 2.5 h for ~90% release). It also increased timolol maleate's corneal retention time (>10 min retention time vs. clear by 10 min) and IOP-lowering capability (~5 h vs. ~3 h duration of action) in rabbits, compared with timolol maleate eye drops. However, no *in vivo* studies with timolol maleate-loaded liposomes were reported by the group [213].

Hao *et al.* explored the potential of a novel hybrid composite system composed of glyceryl monostearate (GMS)-based solid lipid nanoparticles embedded in poloxamer-based thermosensitive hydrogel for ophthalmic delivery using Resina Draconis as a model drug [218]. Incorporation of SLNs into the hydrogel resulted in an increase in the size of the SLNs from 150 to 450 nm, probably due to a sheath around the SLN formed by the poloxamer polymer from the hydrogel that adsorbed on and entangled with the SLN. The size of SLN in hybrid hydrogel system was observed to be stable for 30 days of storage at room temperature. This composite system showed non-irritancy in Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) test. It delayed and decreased the penetration of Resina Draconis across the *ex vivo* albino rabbit cornea in comparison with the SLN alone (lag time 23 vs. 12 min, and permeability coefficient 15.32×10^6 vs. 10.09×10^6 $cm \cdot sec^{-1}$) due to the presence of viscosity enhancer poloxamer in the composite system. Confocal microscopy study showed that coumarin C-6-labeled SLN could penetrate through the cornea and reach the inner corneal endothelial layer in 2 h and continuously accumulated at the endothelial layer till at least 12 h after instillation of the SLN-embedded hydrogel composition system into albino rabbit eyes [218].

3.7.2. Micelle-based composite systems—The use of micelles for drug incorporation in contact lens has so far demonstrated to be more promising for prolonged drug release while maintaining the desired properties of contact lenses than the use of liposomes. The above discussed liposome/lipid nanoparticles based composition systems have not been reported to show more than 5 days' drug release; while non-ionic Brij® surfactant-laden p-HEMA hydrogel contact lenses developed by Chauhan and associates could sustain the release of CsA for up to at least 60 days, depending on the type and concentration of the surfactants, without compromising the mechanical and optical properties of the contact lens [221, 222]. In addition, the sustained release duration depended on drugs' properties too. For example, dexamethasone and dexamethasone 21 acetic acid derivatives could not be brought into sustained release by the same non-ionic Brij (Brij-78) surfactant-laden p-HEMA contact lenses used for the successful sustained release of CsA due to the poor partition of these molecules inside surfactant micellar aggregates [221, 222]. For anionic dexamethasone phosphate, cationic surfactant cetalkonium chloride was used to encapsulate the drug and then this was dispersed in p-HEMA contact lenses with 10% surfactant loading [226]. The *in vitro* release of dexamethasone phosphate from the composition system was prolonged for 50 h, 24 times longer than the unmodified p-HEMA contact lenses. Furthermore, the

composite system also improved the wettability and protein binding of the contact lens [226]. For dexamethasone acetate, silica shell crosslinked methoxy PEG-block-PCL micelles (rod-like morphology) were used to encapsulate the drug and then this was dispersed in poly(2-hydroxyethyl methacrylate-methacrylic acid-ethylene glycol dimethacrylate) hydrogel soft contact lenses. This composite system could sustain the release of dexamethasone acetate for up to 30 days while maintaining the optical transparency of the contact lens [227].

3.7.3. Polymeric or other nanoparticle-based composite systems—Researchers have focused on polymeric nanoparticles using biodegradable and nonbiodegradable polymers such as poly(ethylene glycol dimethacrylate) (PEGDMA), poly(propoxylated glyceryl triacylate) (PPGT), pullulan, PCL, chitosan and albumin to develop therapeutic contact lenses to treat eye diseases [211, 214, 217, 228, 231]. Jung *et al.* reported a p-HEMA contact lens containing timolol-covalently-bound nanoparticles (size-3.5 nm) made of EGDMA and PGT. The contact lens could slowly release timolol for 2–4 weeks due to the hydrolytic cleavage of the ester bond between timolol and the nanoparticles [211]. The timolol release was found to be first order kinetics with rate constant depending on temperature ranging from 25–100 °C; the rate constant varied directly with temperature, as expected from the Arrhenius equation. Under refrigerated storage conditions, the kinetic rate constant was observed to be low, so that timolol was retained in the lens to prevent premature timolol release before the contact lens was put in. Despite these promising results, the composite system had drawbacks of decreasing the water content and oxygen permeability of the contact lens with increasing the loading of the nanoparticles and could not be used for extended wear [211]. In a similar way, Jung *et al.* reported that timolol-loaded PGT nanoparticles dispersed in extended wear silicone hydrogel contact lens could sustain the release of timolol for one month *in vitro* and the release kinetics depended on temperature; but negative impact on the critical properties of the lens was observed, including water content, modulus, and ion and oxygen permeability with increasing the loading of the nanoparticles in the contact lens [217]. Preliminary *in vivo* studies in beagle dogs demonstrated safety and efficacy of this composite system in lowering IOP for 4 days. However, no *in vitro* release or *in vivo* studies with either drug-loaded contact lenses or drug-loaded nanoparticles were reported by the group [217]. Garhwal *et al.* prepared ciprofloxacin-loaded core-shell nanospheres made of copolymer of pullulan and PCL; and then incorporated the nanospheres into a conventional, transparent p-HEMA contact lens for the potential treatment of bacterial keratitis [231]. Depending on their thickness, the lenses exhibited antibacterial activity for up to 3 days due to sustained release of ciprofloxacin that efficiently inhibited the proliferation of bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* [231]. Hashemi Nasra *et al.* synthesized hybrid nanoparticles by crosslinking PCL (hydrophobic inner core), HEMA (hydrophobic inner shell), and PEG-diacrylate (hydrophilic outer shell) and encapsulated the anti-inflammatory drug loteprednol etabonate into the nanoparticles [214]. The drug-loaded nanoparticles were dispersed in poly(HEMA-*co*-N-vinylpyrrolidone) hydrogel contact lenses. The drug release rate of the composite system (~56% in 290 h) is slower than those of the drug-loaded nanoparticles (~52% in 100 h) and hydrogel containing free drug (~55% in 50 h), indicating that incorporating nanoparticles in the hydrogel further retards the drug release. The hybrid system was found

to be nontoxic *in vitro* on rabbit corneal epithelial cells [214]. Behl *et al.* incorporated negatively charged dexamethasone drug into positively charged chitosan (low MW 20–300cP) nanoparticles, and then loaded them into PHEMA based contact lenses [228]. The obtained composite system had similar optical property as the blank contact lens without the nanoparticles, and showed sustained release of dexamethasone *in vitro* with ~56 % drug release in 22 days and ~10% initial burst [228].

Besides the above mentioned drug-loaded nanoparticles, some other nanoparticles, such as silver nanoparticles and meloxicam nanocrystals, which directly showed therapeutic effects, were incorporated in contact lenses to fabricate therapeutic contact lenses. Bazzaz *et al.* reported that silver nanoparticles, loaded into a proxy for soft hydrogel contact lenses, showed excellent antimicrobial activity against *P. aeruginosa,* relative to the *S. aureus*, and exhibited the activity over a period of 6 – 72 h depending on the concentration of the silver nanoparticles and bacterial species. They envisioned that the contact lenses prepared from the silver nanoparticle-laden hydrogel material could provide enough antibacterial effect to lower the risk of microbial-related unfavorable events for lens wearers. However, no study about the effects of nanoparticle-loading on the properties of the contact lens was reported by the group [224]. Zhang *et al.* prepared meloxicam nanocrystals-laden contact lenses for the treatment of post-cataract endophthalmitis [225]. Aggregates of meloxicam nanocrystals at ~100 nm were first coated with bovine serum albumin to lower the ocular irritancy of meloxicam and then dispersed in p-HEMA contact lenses to achieve 5 day release of meloxicam. The meloxicam release kinetics depended on the thickness and degree of crosslinking of the contact lenses. *In vivo* ocular irritation studies using the Draize test on New Zealand white albino rabbits demonstrated that the meloxicam nanocrystals-laden contact lenses were significantly less irritating to the ocular tissues when compared with the marketed drug solution alone [225]. Yavuz *et al.* prepared CsA-loaded PLGA 85:15 or PCL (MW 30,000–70,000 Da) nanoparticles by o/w emulsification solvent evaporation technique and then incorporated the nanoparticles into PCL implant/fiber composite system by electrospinning/implant molding method [232]. They found that CsA was present in cornea, sclera and lens even 90 days after subconjunctival implantation of the composite systems in dry-eye induced mice. The fiber composite system had better CsA tissue concentration and healing process than the implant composite system [232]. Yang *et. al.* also used PLGA nanoparticles (113–187 nm) to load hydrocortisone butyrate and then suspended them in a thermosensitive PLGA-PEG-PLGA gel. They used surfactants chitosan, pluronic and PVA to prepare the nanoparticles, and found that the nanoparticles made of chitosan (187 nm size and +31 mV zeta potential) were taken up by human corneal epithelial cells more than the nanoparticles made of PVA (164 nm size and −20 mV zeta potential) or pluronic (113 nm size and −26.9 mV zeta potential). The nanoparticle-laden gel composite system released hydrocortisone slower (~100% in 28–32 days, no burst) than both the drug-loaded nanoparticles (~70% in 1 day and ~80% in 16 days) and gel (~45% in 24 h and ~85% in 24 days). Ibrahim *et al.* evaluated the stability of nanoparticles made of chitosan, sodium alginate, PCL, PLA and PLGA under accelerated stability testing methods [110]. They found that the nanoparticles made of chitosan and PCL were more stability and had longer shelf life than the nanoparticles made of the other materials. They also found that when composite systems made of two types of nanoparticles dispersed in HPMC gel could

significantly increase the ocular bioavailability of celecoxib by ~4.8–29.7-fold in comparison to the drug suspension. The chitosan-nanoparticles-laden gel increased the ocular bioavailability of celecoxib more than the PCL-nanoparticles laden gel due to the stronger mucoadhesive and penetration enhancement properties of chitosan [110].

Table 14 summarizes various nanoparticle-based composite systems and their characteristics.

4. Safety and regulatory considerations

Nanomaterials can exhibit altered physical or chemical properties or biological effects (dimension-dependent properties or phenomena) in comparison to their corresponding large-scale materials with the same chemical composition [233–236]. Each class of nanoparticles is unique, and "FDA believes that evaluations of safety, effectiveness, public health impact, or regulatory status of nanotechnology products should consider any unique properties and behaviors that the application of nanotechnology may impart" [233]. There are three major considerations related to the products based on nanotechnology, which includes characterization, safety, and environmental impact [237]. Various essential parameters that need to be considered during the characterization of nanoparticles are: critical physical and chemical properties and their impact on quality and performance of the product, reliable and reproducible *in vitro* and *in vivo* characterization tools or techniques and their sensitivity, short/long-term stability issues in various environments, critical quality attributes, critical processing variables, overall chemistry, manufacturing, and controls (CMC), and the forms in which they were presented to the host [237]. The important parameters that need to be considered for evaluating the *in vivo* safety of nanoparticles are: tissue penetration/distribution and pharmacokinetics parameters (ADME profile) of the nanomaterials compared to their large-scale counterparts, tissue retention, transient and/or permanent bioeffects *in vivo*, clearance mechanisms, and potential variation in effects in different tissues [238]. The major parameters that need to be considered for assessing the environmental impact of the nanomaterials are: potential implications of the releasing of these materials into the environment post use, and methodologies to detect and quantify the environmental effects [237]. In June 2014, FDA released the final guidance for industry related to nanomaterials – "*Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology*" [233]. Currently, FDA addresses the issues related to the safety, effectiveness, and public health impact of nanotechnology products on a case by case basis, and advises industry to consult with the agency in the early development process [233]. Therefore, any entity that plans to develop a nanoparticle product for ophthalmic applications, should follow the FDA guidelines and communicate with FDA as early as possible when it is feasible to learn the regulatory requirements and implement appropriate steps for bringing the product into the market.

5. Summary and perspectives

Nanoparticles have shown significant promise for effective drug delivery to the anterior segment of the eye. Over the past few years, increasing progress has been occurring in this area and can be further improved for clinical translation. Current development of

nanoparticles for anterior eye segment drug delivery mainly focuses on the route of topical administration. Although the subconjunctival administration is well studied for implants, it is scarcely studied for nanoparticles in the literature. In addition, systemic and especially intracameral administrations were viewed as possibilities for drug delivery to the anterior eye segment. To date, there is no nanoparticle that has been designed to utilize these two routes of administration. We think that more studies on the effectiveness of subconjunctival, intracameral and systemic routes for delivering drugs to the anterior segment of the eye are needed and will be beneficial. Most of the nanoparticles for drug delivery to the anterior eye segment are evaluated in terms of their physicochemical properties, drug loading capability, *in vitro* drug release and toxicity, and comparatively simple *in vivo* tests. With the array of polymers and fabrication techniques currently available, nanoparticles can be engineered in a way that makes them safer, more stable, and easier to prepare; provide better control over drug release; and have the capability to deliver not only small molecules, but also peptides/proteins and genes. With the choice of a wide range of biodegradable polymers that have emerged, there is an increased versatility in the formulation of nanoparticles in terms of category and function. We envision that more innovative, functional, practical and safe nanoparticles, including nanoparticle-loaded therapeutic contact lenses, will be continuously developed to effectively deliver drugs to the anterior segment of the eye. During future nanoparticle development, several key areas of study need to be addressed, including active targeting, pharmacokinetic/pharmacodynamics (PK/PD), safety and toxicology, stability, and production scale-up.

Active targeting based on the binding affinity between a targeting ligand attached on the nanoparticle surface and the targeted units of diseased tissue (receptors/antigens) has been considered as an effective approach for improving the efficacy of nanoparticle-based delivery systems [239, 240]. Since polymers can offer many reactive functional groups, active targeting to specific tissues in the anterior eye segment is expected to be developed by conjugating targeting moieties onto the polymeric nanoparticle surface in the near future. However, as the active targeting is a complex process, the following factors need to be considered thoroughly during the nanoparticle development for active targeting: targeted unit localization and expression in the diseased site, targeting ligand selection, conjugation chemistry, ligand density, nanoparticle size and architecture, accessibility of the nanoparticles to the target site, non-specific binding, and *in vivo* stability of the ligand-conjugated nanoparticles [239, 241–244].

As nanoparticles can alter the PK/PD properties of a free drug and subsequently the therapeutic effect and toxicity of the drug [182], there is an unmet need to critically assess the PK, and local and systemic biodistribution of the nanoparticles after administration into the eye. It is also in need to study how ocular diseases affect anterior segment nanodelivery. In addition to the PK/PD studies, more focused emphasis should be given to study the acute and long-term toxicity of the nanoparticles, and the ultimate fate of the nanoparticles or their degradants after the release of the drug at the intended site. More deeply, it is important to understand and measure the effects of nanoparticulate parameters (size, charge, architecture, surface chemistry, active targeting) on the *in vivo* fate including clearance, and the long-term toxicity of the nanoparticles. Future studies can also be performed in evaluating the specific interactions of the nanoparticles with cells, tissues and/or human organs, and the

mechanisms for the nanoparticles to enhance the drug bioavailability in the anterior eye segment.

Physical and chemical stability is another key aspect that needs to be considered during the pharmaceutical development of drug-nanoparticulate systems to ensure the safety, efficacy, and quality of the products [165, 245]. Currently, there are scarce studies about the stability of nanoparticles for ocular applications. In the future development, the physical, chemical, biological, and microbiological stability of nanoparticles should be evaluated according to regulatory guidelines. Most of the reported methods for fabricating nanoparticles for ocular applications are at lab scale and not suitable for industry mass production. In the future, cost-effective technology needs to be developed to scale up nanoparticle production for clinical translation. During scale up, special consideration should also be given to the techniques for sterilizing the nanoparticles such as aseptic processing/sterile filtration or terminal sterilization by autoclaving. Besides the above discussed key areas of future studies, the evaluation of the environmental impacts of the nanomaterials needs to be carried out in the future [237]. As nanoparticles are still in their early development stage for treating diseases in the anterior segment of the eye, there is no clinical trial for nanoparticle systems yet. With the further development of nanoparticles for drug delivery and other treatments for the anterior eye segment, appropriately designed clinical trials for the nanoparticle systems are expected to be conducted in the future.

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Abbreviations

PACA	poly(alkyl cyanoacrylate)
CsA	cyclosporine A
IOBA-NHC	spontaneously immortalized epithelial cell line from normal human conjunctiva
HCE	human corneal epithelial cells
FITC	fluorescein isothiocyanate
HA	hyaluronic acid
IOP	intraocular pressure
CH	cholesterol
CS-CH	chlolesterol modified chitosan
PLA	poly(lactide)
PLGA	poly(lactide-*co*-glycoside)
PCL	poly(ε-caprolactone)

GRGDS	Gly-Arg-Gly-Asp-Ser
RGD	Arg-Gly-Asp
BXD	boxed molecular dynamics
BKC	benzalkonium chloride
SLN	solid lipid nanoparticles
RCE	corneal epithelial cells
GMS	glyceryl monosterate
HET-CAM	Hen's Egg Test - Chorioallantoic Membrane
NLC	nanosctructured lipid carriers
PAMAM	polyamidoamine
DPT	dendritic polyguanidilyated translocators
PHEMA	poly(2-hydroxyethyl methacrylate)
PEG	poly(ethylene glycol)
EGDMA	ethylene glycol dimethacrylate
PEGDMA	poly(ethylene glycol dimethacrylate)
PPGT	poly(propoxylated glyceryl triacylate)
VEGF	vascular endothelial growth
PDI	polydispersity index
AUC	the area under curve
MRT	mean residence time
CS-NAC	N-acetyl-L-cysteine functionalized chitosan
PK	pharmacokinetics
PD	pharmacodynamics
HEC	human corneal epithelial cells

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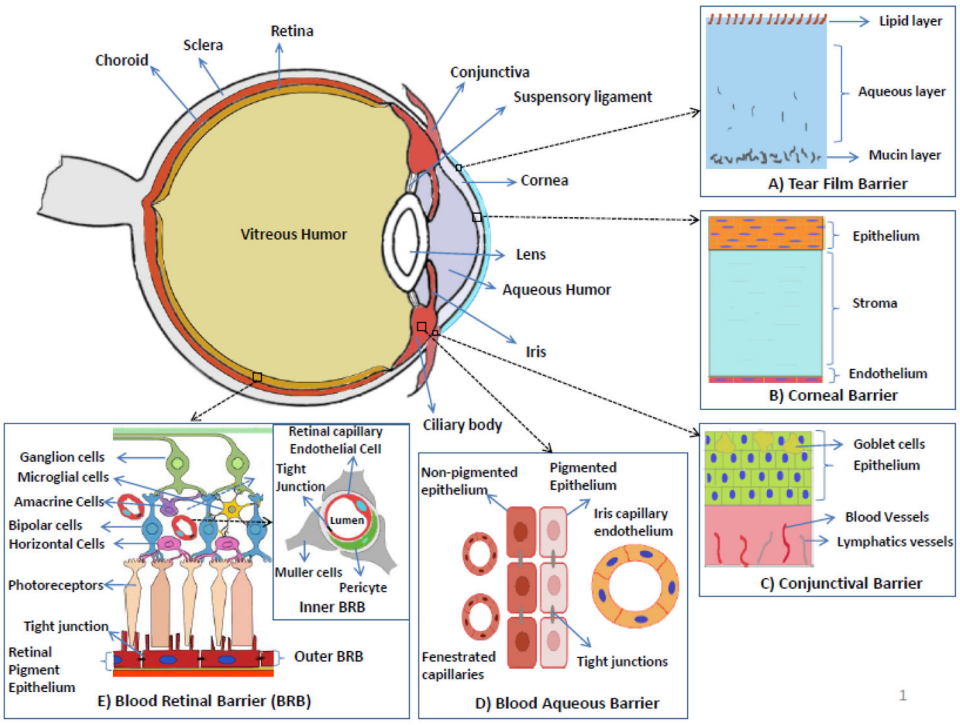


Fig. 1.

Structure of the anterior segment of the eye and ocular barriers for drug delivery. A) Tear film barrier: Main components of the tear film include mucins, water and lipid, and acts a defensive barrier to the foreign-object access to the cornea and conjunctiva. B) Corneal barrier: avascular and comprised of three major layers which are epithelium (multiple layers stacked on each other), stroma and endothelium (single layer). Acts as a barrier preventing the drug absorption from the lacrimal fluid into the anterior chamber after the topical administration. C) Conjunctival barrier: mucous membrane consisting of conjunctival epithelium (2–3 layers thick), and an underlying vascularized connective tissue. Acts a barrier to the topically administered drugs and relatively in-efficient compared to the corneal barrier. D) Blood-aqueous barrier: located in the anterior segment of the eye. Formed by the capillary endothelium in the iris, and the ciliary epithelium which both contain tight junctions. The barrier is relatively inefficient compared to the blood retinal barrier and small molecules can reach the aqueous humor by permeation through fenestrated capillaries in the ciliary processes. E) Blood-retinal barrier (BRB): located in the poster segment of the eye. Formed by the retinal pigment epithelium (outer BRB) and the endothelial membrane of the retinal blood vessels (inner BRB), both contain tight junctions. The tight junctions restrict the entry of the drugs from the blood (systemic) into the retina/aqueous humor.

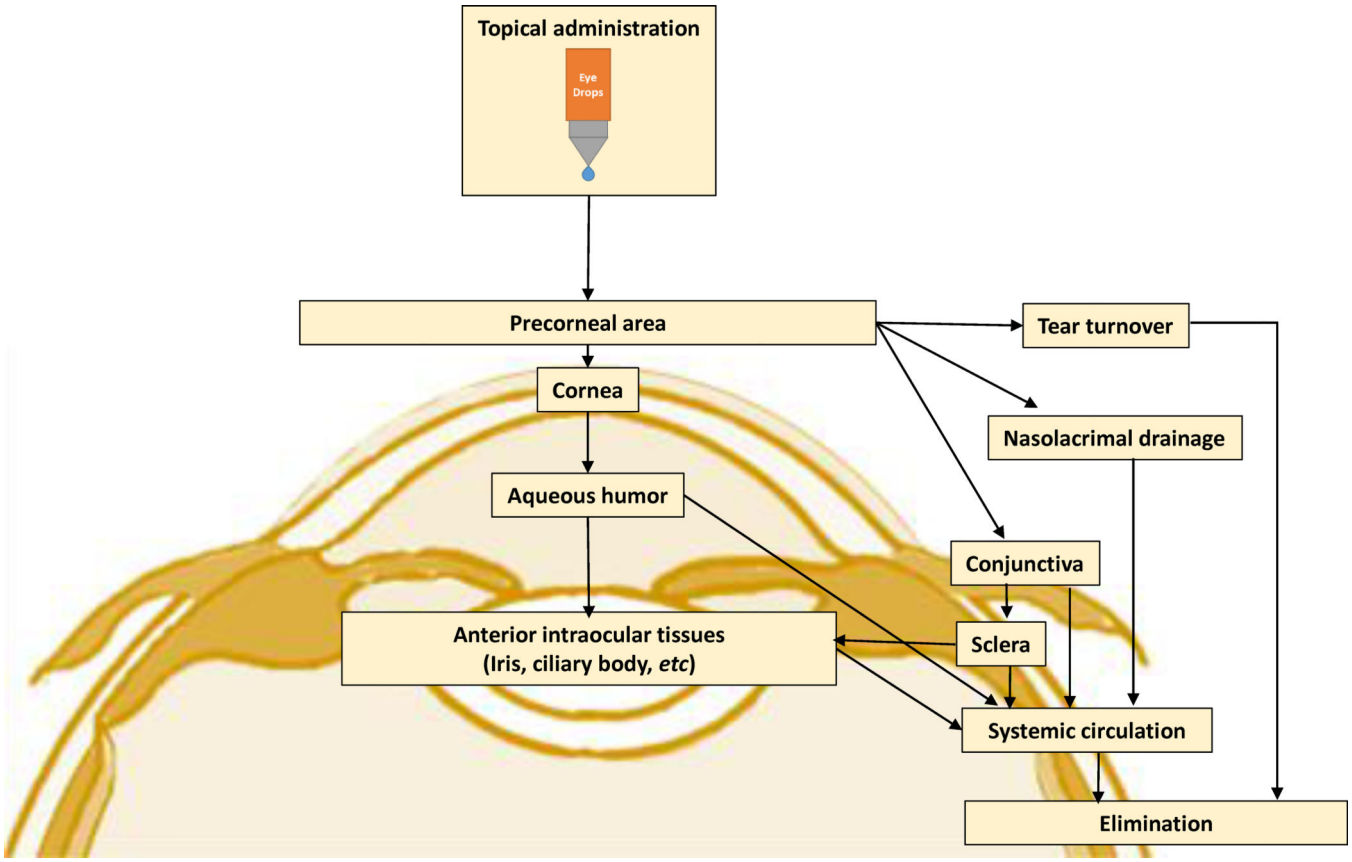


Fig. 2.
Flow chart presenting the drug pathway into the eye after topical application.

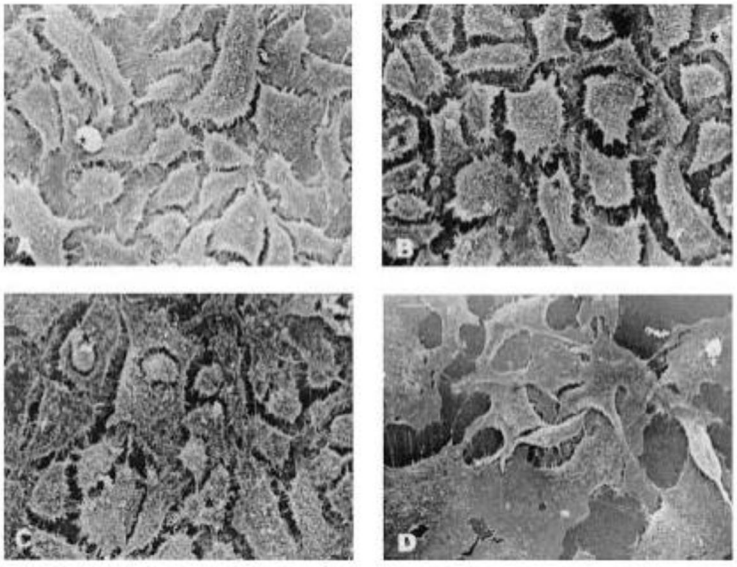


Fig. 3.

Scanning electron microphotographs of Chang cells exposed to culture medium, chitosan nanoparticles, and benzalkonium chloride for 24 h. (A) (Negative control, culture medium) Cells showing abundant microvilli and intact membrane details. (B) (Chitosan nanoparticles, 0.25 mg/ml) cells showing well-preserved morphology, an intact cell surface, and abundant microvilli, as expected for an epithelial cell. (C) (Chitosan nanoparticles, 1 mg/ml). (D) (Positive control, benzalkonium chloride) Cells were flat and showed absence of microvilli and broken membrane. Magnification ×750 (bar =15 μm). [Reprinted from “Pharmaceutical research, Chitosan nanoparticles as new ocular drug delivery systems: *in vitro* stability, *in vivo* fate, and cellular toxicity, 21.5 (2004): 803–810. De Campos, A. M., Diebold, Y., Carvalho, E. L., Sánchez, A., & José Alonso, M. c 2004 Plenum Publishing Corporation” With permission of Springer.]

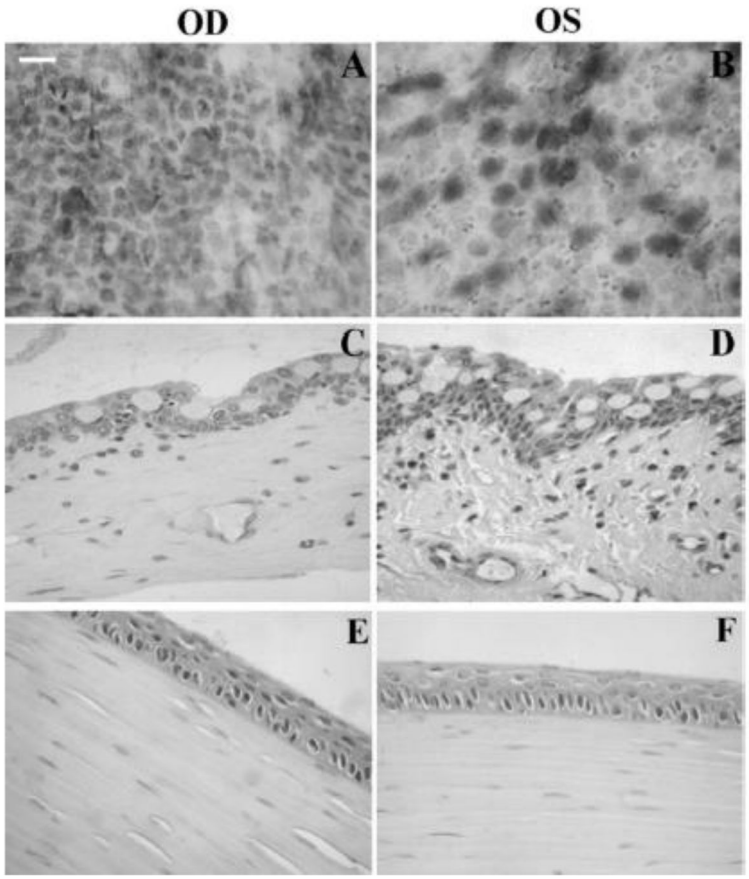


Fig. 4.

Ocular surface structures of CSNP-treated (OD) and control (OS) rabbit eyes. Rabbits were exposed to CSNPs for 24 hours. Representative conjunctival impression cytology (A, B) and conjunctival (C, D) and corneal (E, F) sections are shown. Conjunctival and corneal epithelia from both control and treated eyes displayed normal cell layers and morphology. No signs of tissue edema were observed in any structure studied after exposure to CSNPs compared with controls. Scale bar, 100 μm. [Reprinted from De Salamanca, Amalia Enríquez, et al. "Chitosan nanoparticles as a potential drug delivery system for the ocular surface: toxicity, uptake mechanism and *in vivo* tolerance." Investigative ophthalmology & visual science 47.4 (2006): 1416–1425. Copyright © Association for Research in Vision and Ophthalmology]

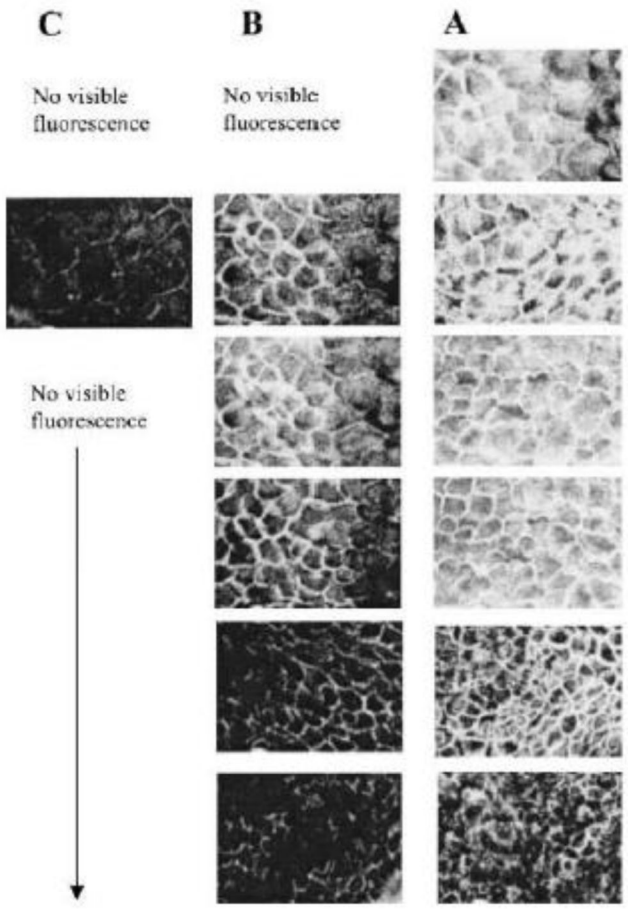


Fig. 5.

Confocal fluorescence images at different levels from the rabbit corneal epithelium (5 μm sequential cross sections from the corneal surface) at 1 h postinstillation of (C) Chitosan-fluorescein nanoparticles, (B) Chitosan-fluorescein solution. (A) image of cross section of a nontreated cornea. Round fluorescent spots corresponding to the chitosan-fluorescein nanoparticles were observed inside the cells. [Reprinted from “Pharmaceutical research, Chitosan nanoparticles as new ocular drug delivery systems: *in vitro* stability, *in vivo* fate, and cellular toxicity, 21.5 (2004): 803–810. De Campos, A. M., Diebold, Y., Carvalho, E. L., Sánchez, A., & José Alonso, M. c 2004 Plenum Publishing Corporation” With permission of Springer.]

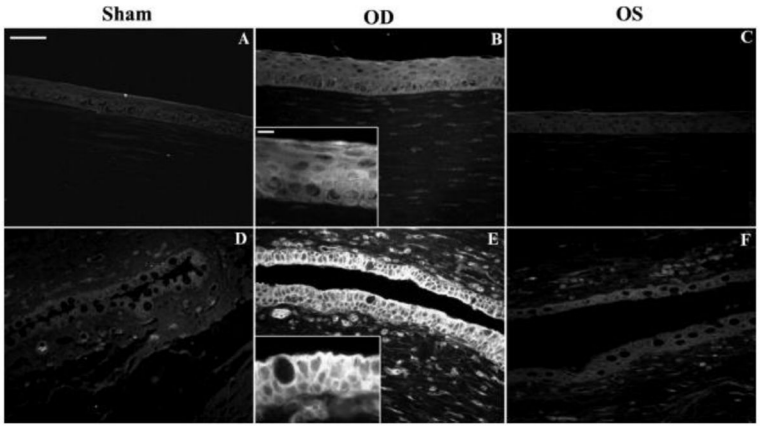


Fig. 6.

Chitosan nanoparticle *in vivo* uptake. Fluorescence microscopy of ocular surface structures of sham-treated (A, D), CSNP-treated (B, E), and contralateral control (C, F) rabbit eyes. Representative corneal (A–C) and conjunctival (D–F) sections are shown. No fluorescence was detected in sham control corneas (A) or conjunctivas (D). (B) Corneal epithelial cells of CSNP-treated rabbits were uniformly fluorescent. (B, inset): enlargement showing a detail of corneal epithelial fluorescence pattern. (E) Fluorescence in conjunctival epithelial cells was intense in apical cell membranes and positive along the basolateral cell membrane. (E, inset) Enlargement showing the basolateral membrane fluorescence staining in goblet and non–goblet cells. (C, F) Some fluorescence was detected in corneal and conjunctival epithelial cells from contralateral control eye (OS), although much less intense than in the treated (OD) eye. Scale bar (A–F) 50 μm; insets: 10 μm). The *in vivo* uptake by conjunctival and corneal epithelia was confirmed from these fluorescence microscopy images of eyeball and lids sections confirmed. [Reprinted from De Salamanca, Amalia Enrique, et al. “Chitosan nanoparticles as a potential drug delivery system for the ocular surface: toxicity, uptake mechanism and *in vivo* tolerance.” Investigative ophthalmology & visual science 47.4 (2006): 1416–1425. Copyright © Association for Research in Vision and Ophthalmology.]

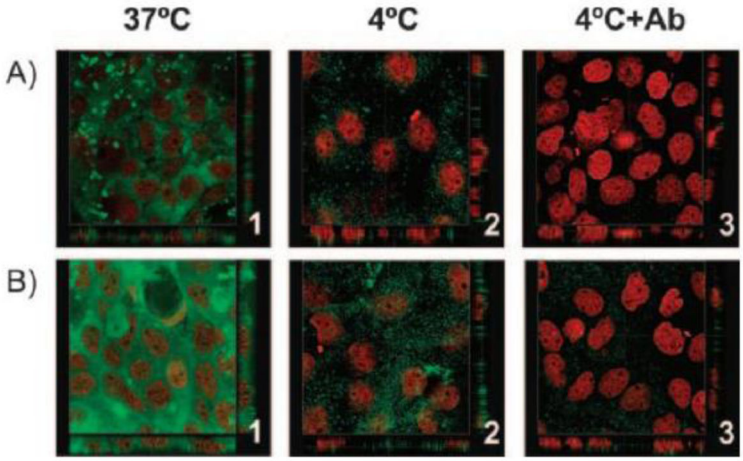


Fig. 7.

Confocal images showing the internalization of (A) Hyaluronic acid: Chitosan oligomer (mass ratio, 1:2), and (B) Hyaluronic acid: Chitosan (mass ratio, 2:1) NP in HCE cells. The NPs were incubated at 37°C (1), 4°C (2), and 4°C after blocking of the CD44 receptor with the monoclonal antibody Hermes-1 (3). The images show cross sections in the x–y and x–z axes of the series. HA was labeled with fluoresceinamine (green), and the cell nuclei were stained with propidium iodide (red). Magnification, 63. An extensive internalization of nanoparticles was observed at 37°C evidencing endocytic uptake of the nanoparticles into the cells (B1). The observed minor cell association at 4°C could be attributed to the receptor-mediated uptake of the particles (B2). However, when the study was repeated at 4°C in presence of CD44 receptor blocker (monoclonal antibody Hermes-1), a negligible uptake of nanoparticles was observed (B3), thus concluding the internationalization mechanism as CD44 receptor-mediated endocytosis. [Reprinted from de la Fuente, Maria, Begona Seijo, and Maria J. Alonso. “Novel hyaluronic acid-chitosan nanoparticles for ocular gene therapy.” Investigative ophthalmology & visual science 49.5 (2008): 2016–2024. Copyright © Association for Research in Vision and Ophthalmology]

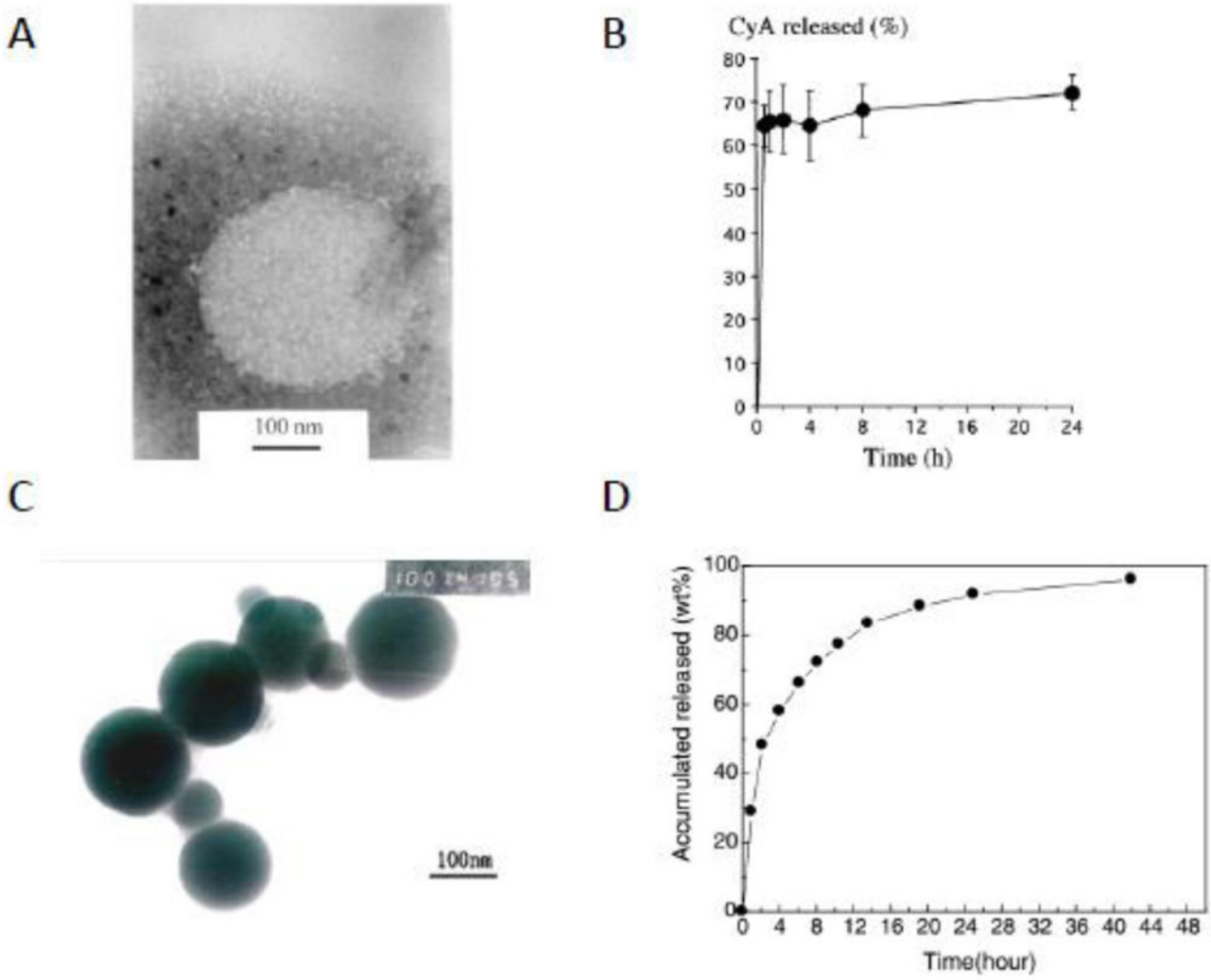


Fig. 8.

Transmission electron micrograph of the CsA-loaded CS nanoparticles (prepared by ionotropic gelation method) (A). *In vitro* CsA release profile from CyA-loaded CS nanoparticles (B) [Reprinted from International journal of pharmaceutics, 224(1), (2001). De Campos, A. M., Sánchez, A., & Alonso, M. J. Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporine A. 159–168. Copyright © 2001 Elsevier Science B.V., with permission from Elsevier]. TEM micrographs of cholesterol modified chitosan nanoparticles (formed by self-aggregation) (C). *In vitro* CsA release from CS-CH self-aggregated nanoparticles (D) [Reprinted from Carbohydrate Polymers 65.3 (2006). Yuan, X. B., Li, H., & Yuan, Y. B. Preparation of cholesterol-modified chitosan self-aggregated nanoparticles for delivery of drugs to ocular surface. 337–345 Copyright © 2006 Elsevier Ltd, with permission from Elsevier.]

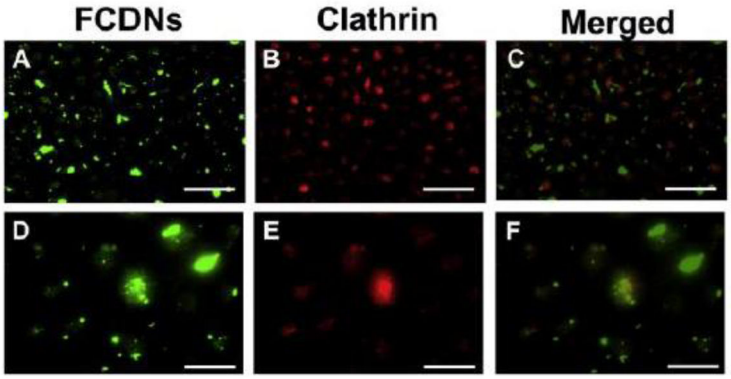


Fig. 9.

Uptake of FITC labeled Chitosan-Dextran sulfate Nanoparticles (FCDNs) occurs by a clathrin-dependent mechanism. (A, D) Images taken incubation with of cells FCDNs for 30 min at 37 C. (B, E) Cells are stained with clathrin (red). (C, F) Merged image showing the colocalization of FCDNs and clathrin (i.e., yellow spots). Magnification: (A–C) ×20; (D–F) ×63. Scale bar: (A–C) = 50 μm; (D–F) = 20 μm. Images shown are typical results after 3 independent trials. [Reprinted from Colloids and Surfaces B: Biointerfaces 149 (2017). Chaiyasan, W., Praputbut, S., Kompella, U. B., Srinivas, S. P., & Tiyaboonchai, W. Penetration of mucoadhesive chitosan-dextran sulfate nanoparticles into the porcine cornea. 288–296. © 2016 Elsevier B.V. with permission from Elsevier.]

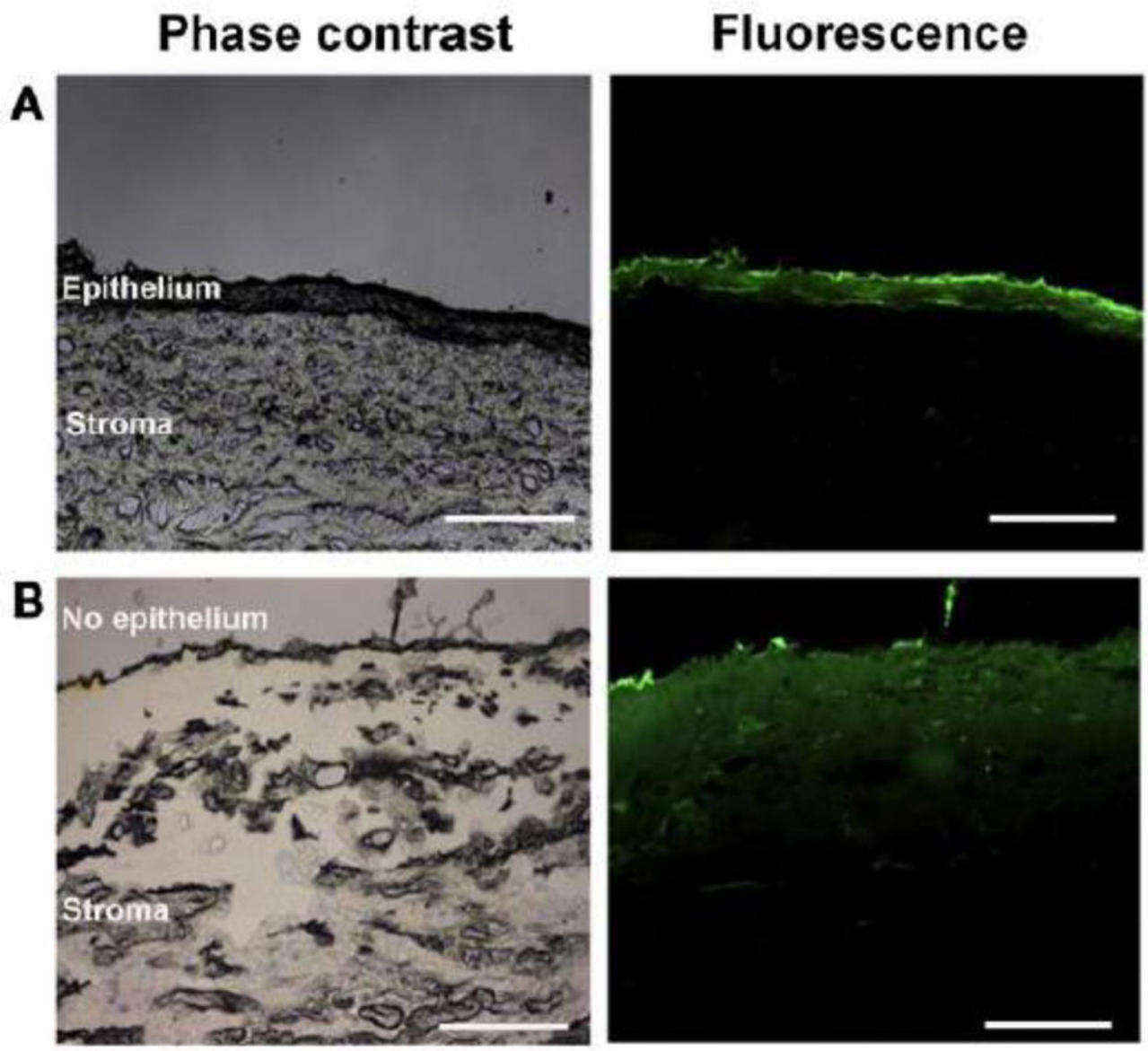


Fig. 10.

Cross section of the porcine cornea after its exposure to FCDNs for 3 h. (A) Intact corneal epithelium. (B) After removal of the corneal epithelium. Scale bar = 250 μm. Images shown are typical results after 3 independent trials. [Reprinted from Colloids and Surfaces B: Biointerfaces 149 (2017). Chaiyasan, W., Praputbut, S., Kompella, U. B., Srinivas, S. P., & Tiyaboonchai, W. Penetration of mucoadhesive chitosan-dextran sulfate nanoparticles into the porcine cornea. 288–296. © 2016 Elsevier B.V. with permission from Elsevier.]

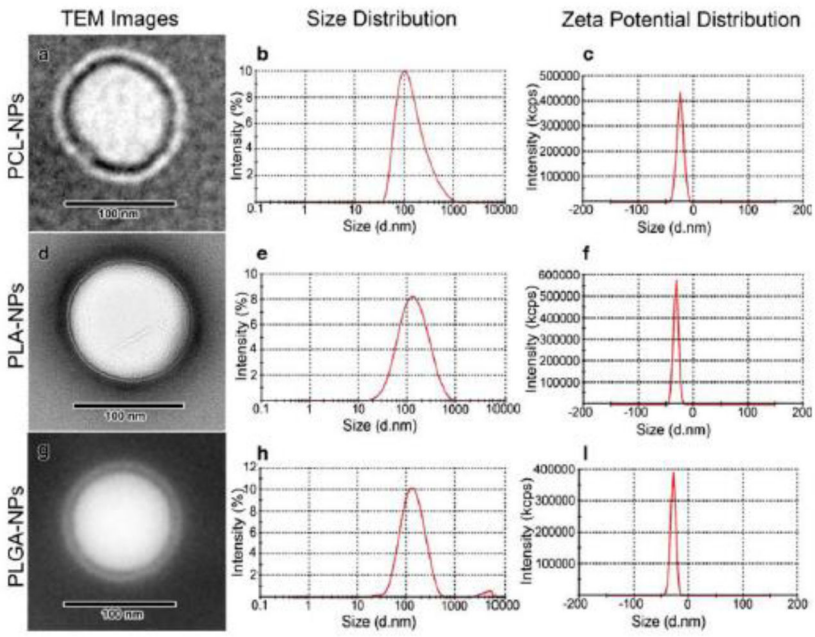


Fig. 11.

TEM images, size and zeta potential distribution curves for brimonidine-loaded nanoparticles. TEM image (a), size distribution curve (b) and zeta potential distribution curve (c) of PCL-nanoparticles, TEM image (d), size distribution curve (e) and zeta potential distribution curve (f) of PLA-nanoparticles, TEM image (g), size distribution curve (h) and zeta potential distribution curve (i) of PLGA-nanoparticles. [Reprinted permission "Pharmaceutical research. Novel topical ophthalmic formulations for management of glaucoma. 30.11 (2013): 2818–2831. Ibrahim, M. M., Abd-Elgawad, A. E. H., Soliman, O. A., & Jablonski, M. M. © Springer Science+Business Media New York 2013." With permission of Springer]

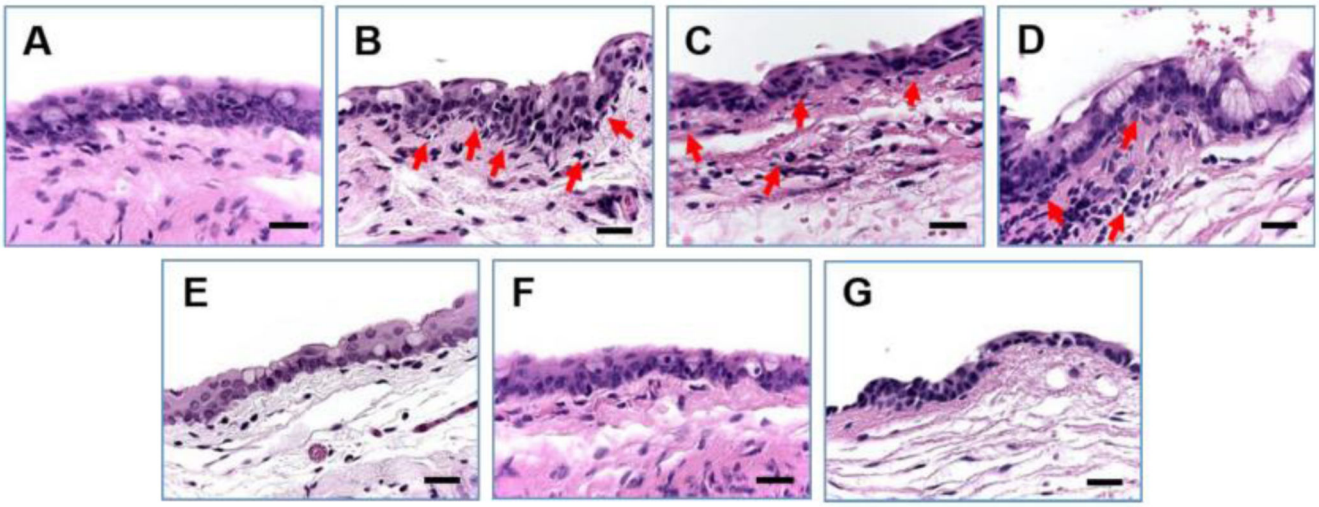


Fig. 12.

Histopathology analysis of ocular tissues of mice after seven different treatment types: (a) healthy, experimental dry eye treated with (b) Saline (x1/week), (c) Saline (x2/week), (d) Blank nanoparticles, (e) nanoparticle+ CsA (x1/week), (f) nanoparticle+CsA (x2/week), and (g) RESTASIS (x3/day). The scale bars (black) are 300 μmin length. The arrows (red) represent some of the inflammatory infiltrates such as lymphocytes, polymorphonuclears and eosinophils observed. [Reprinted from “Pharmaceutical research, Phenylboronic acid modified mucoadhesive nanoparticle drug carriers facilitate weekly treatment of experimentally induced dry eye syndrome, 8(2), (2015) 621-635. Liu, S., Chang, C. N., Verma, M. S., Hileeto, D., Muntz, A., Stahl, U., Woods, J., Jones, W., Gu, F. X. © Tsinghua University Press and Springer-Verlag Berlin Heidelberg 2014” With permission of Springer.]

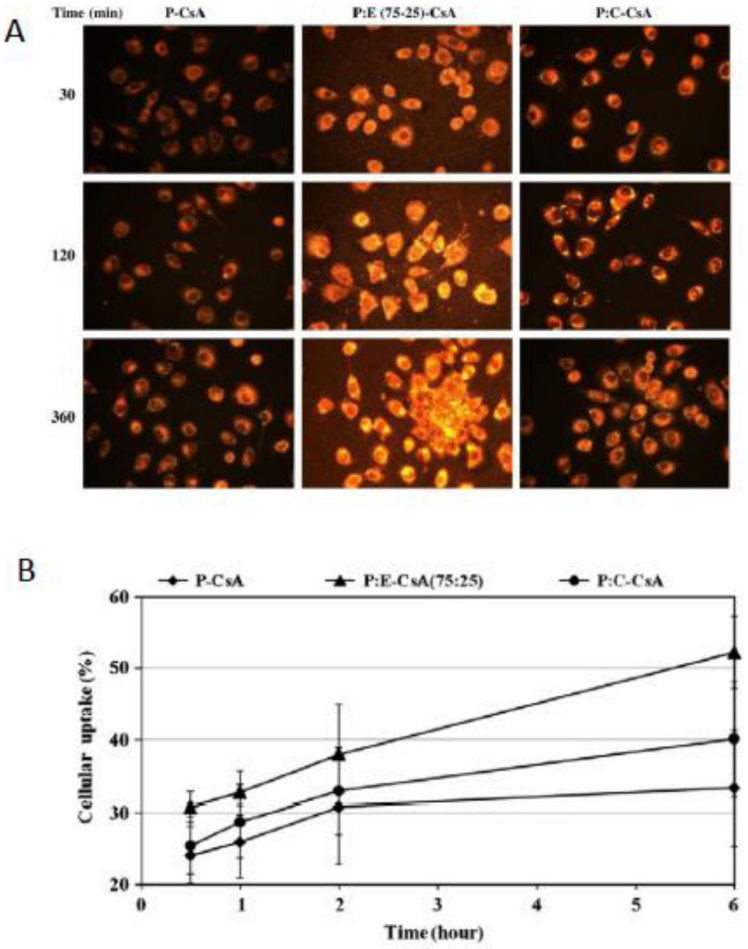


Fig. 13.

Time-dependent nanoparticles uptake by L929 (mouse fibroblast) cells. Photographs of representative series of cells exposed to 0.5 mg/mL NPs for 30, 120 and 360 min. Original magnification 400× (A). Cellular uptake efficiency (%) of Nile red labeled nanoparticles (B). [Reprinted from Journal of controlled release 151.3 (2011). Aksungur, P., Demirbilek, M., Denkbaş, E. B., Vandervoort, J., Ludwig, A., & Unlu, N. Development and characterization of Cyclosporine A loaded nanoparticles for ocular drug delivery: Cellular toxicity, uptake, and kinetic studies. 286–294. Copyright © 2011 Elsevier B.V. with permission from Elsevier.]

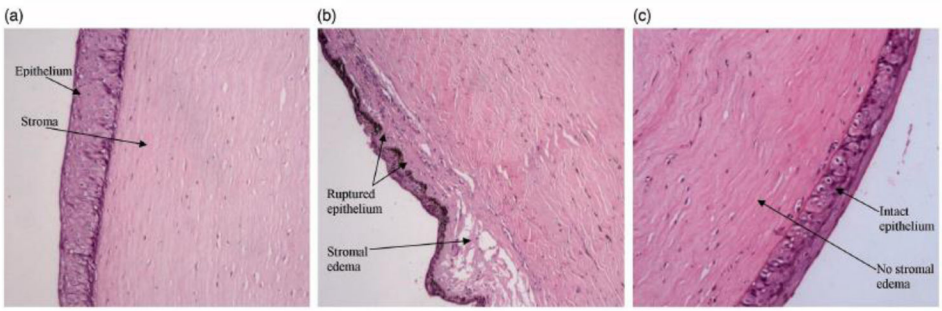


Fig. 14.

Images of histopathology of isolated cornea after treatment with (a) Control (0.9% NaCl), (b) Irritant (0.1N NaOH) and (c) SLN. Irritant treated cornea showed a severely injured epithelial layer, which was separated from the Bowman's layer at various regions of the cornea, and showed a significant corneal swelling. The control and SLN-treated cornea displayed minimal or insignificant swelling. [Reprinted form Kumar, Rakesh, and Vivek Ranjan Sinha. "Solid lipid nanoparticle: an efficient carrier for improved ocular permeation of voriconazole." Drug development and industrial pharmacy 42.12 (2016): 1956–1967. © 2016 Informa UK Limited, trading as Taylor & Francis Group. With permission from Taylor & Francis Ltd (www.tandfonline.com)]

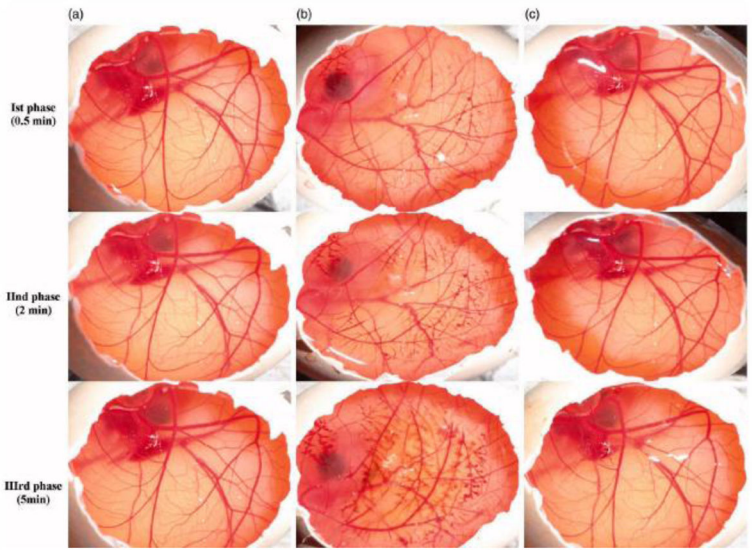


Fig. 15.

Images of HETCAM assay after treatment with (a) Control (0.9% NaCl), (b) Irritant (0.1N NaOH) and (c) SLN. Irritant treated showed a significant lysis, hemorrhage, and coagulation while the control and SLN-treated displayed non-irritant property. [Reprinted from Kumar, Rakesh, and Vivek Ranjan Sinha. “Solid lipid nanoparticle: an efficient carrier for improved ocular permeation of voriconazole.” Drug development and industrial pharmacy 42.12 (2016): 1956–1967. © 2016 Informa UK Limited, trading as Taylor & Francis Group. With permission from Taylor & Francis Ltd (www.tandfonline.com)]

Table 1

Administration routes for delivering drugs to the anterior segment of the eye.

Route of administration	Advantages	Limitation
Topical	simple, convenient, self-administrable and noninvasive administration; avoiding the blood-aqueous barrier; no first-pass metabolism	short contact time of drug on the ocular surface; low efficiency and low bioavailability due to corneal and conjunctival barriers, tear clearance, and nasolacrimal drainage
Intracameral	avoiding the cornea, conjunctiva and blood-aqueous barrier; no first-pass metabolism; high efficacy; high bioavailability	usually need reconstitution; correct dosing and preparation are critical.
Subconjunctival	easy and minimally invasive administration; avoiding the cornea and blood-aqueous barrier; no first-pass metabolism; good efficacy; good bioavailability; sustained release	conjunctival blood and lymphatic clearance
Systemic	convenient to deliver a large dose of the drug; noninvasive; avoiding the cornea	low bioavailability due to systemic absorption and blood-aqueous barrier; first-pass metabolism

Table 2

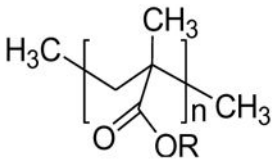
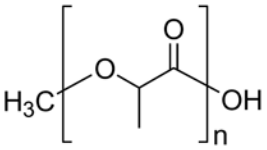
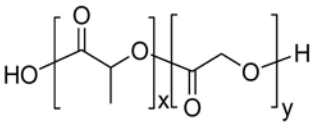
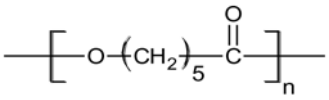
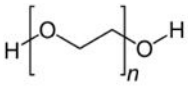
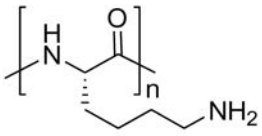
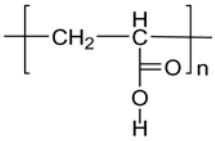
Summary of the techniques used for physicochemical and *in vitro* and *in vivo* characterizations of nanoparticles for anterior ocular delivery.

Property	Characterization Method
Yield	Gravimetric [53]
Particle size/morphology	Dynamic light scattering [25, 53, 91, 95, 118, 119, 123, 142, 150, 171, 174, 178, 185, 193, 194, 210, 213, 246–255]
	Transmission electron microscopy (TEM) [30, 91, 102, 113, 118, 119, 174, 178, 191, 193, 194, 208, 248, 256–260]
	Scanning electron microscopy (SEM)[117, 119, 218, 251, 253, 261, 262]
	Field Emission Scanning Electron Microscope [210]
	Thermomicroscopy [263]
	Atomic microscopy [228]
Zeta potential	Laser Doppler anemometry (LDA)/electrophoretic mobility [25, 95, 119, 123, 142, 174, 185, 248, 249, 252–255]
Chemical characterization	Fourier Transform Infrared (FTIR) spectroscopy [91, 95, 113, 117, 142, 208, 259, 261, 263–265]
	Nuclear magnetic resonance (NMR) spectroscopy [203, 208, 259, 264]
	Mass spectrometry [266]
	Elemental analysis [251, 264]
Solid-state characterization	Infrared spectrophotometry [142, 261, 263]
	X-ray diffractometry [91, 113, 142, 171, 263, 267]
	Differential scanning calorimetry (DSC) [91, 95, 113, 142, 171, 174, 191, 193, 218, 259, 263, 267]
	Thermal gravimetric analysis (TGA) [261]
Molecular weight characterization	Size exclusion chromatography [203]
Surface tension	Tensiometer [203]
Viscosity of solution	Rheometer/viscometer [185, 203, 260, 265, 268]
Drug loading and Entrapment efficiency	HPLC [25, 89, 171, 174, 193, 194, 208, 221, 249, 250, 254]
	UV-Vis spectroscopy [91, 102, 113, 117–119, 178, 210, 253, 255, 258, 260, 262, 264, 267]
	Liquid scintillation counter for radio labelled[123]
	bioassay [252]
	GPC and UV [213] (drug separated by dialysis or centrifugation or membrane filtration)
In vitro drug release	Sample and Separate [25, 95, 117, 119, 123, 142, 150, 210, 269]
	Dialysis system [102, 113, 150, 178, 191, 193–195, 208, 246–249, 253, 255, 259–263, 265, 267]
	Diffusion cell [171, 213, 268]
	(drug analysis by spectrofluorometric or UV-Vis or HPLC or LC-MS/MS or liquid scintillation counting)
Drug stability	HPLC [208, 210, 218, 265, 270]
Enzyme stability	Incubation with Mucin ad Lysozyme and analyze for particle size, Zeta and drug leakage [117, 256]
	Incubation in simulated tear fluid and analyze for particle size [252]
Storage stability	Incubation at appropriate conditions and analyze for particle size, poly-dispersity index, zeta potential and/or drug leakage [30, 142, 179, 208, 255, 261, 262, 265, 271]

Property	Characterization Method
In vitro cytotoxicity	MTT colorimetric assay or XTT colorimetric assay [102, 230, 252] [30]
	SEM [256]
	Neutral red (NR) assay[174]
	Erythrocyte morphology & Hemolysis/spectrophotometer[208]
	Resazurin assay[258]
In vitro irritancy test	Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) test [113, 178, 218, 258, 259, 262, 267, 268]
Mucoadhesive capacity	Mucin-particle method (incubating with mucin-centrifugation-analyze free mucin by colorimetric method) [91, 113, 185, 258, 259, 267]
	Ex vivo study by fluorescence microscopy [117]
In vitro ocular permeability	Cell Monolayers – Transwell Inserts [171, 174] (drug analysis by HPLC or LC-MS/MS)
Ex vivo ocular permeability	Excised cornea and a diffusion apparatus [30, 178, 191, 193–195, 213, 254] (drug analysis by HPLC or LC-MS/MS)
Ex vivo tissue hydration	Gravimetric -Wet and dry weight (desiccation) [178, 213]
Cellular internalization or uptake (*in vitro* or *ex vivo*)	Immunofluorescence Assays by confocal laser scanning microscope [113, 174, 178, 218, 247, 252, 256, 272]
	Spectrofluorimetry [256, 272]
	Fluorescence microscopy [230, 272]
	Flow cytometry [128]
In vivo ocular tolerability	Modified Draize Test using a slit-lamp[25, 142, 193, 273]
	Histopathology [30, 150, 194]
	Slit lamp examination of clinical observations and ocular reactions (such as swelling and redness, conjunctival chemosis, discharge, iris and corneal lesions)-ocular irritation scoring testing system [150, 185, 203, 208, 213, 252, 254, 255, 272]
In vivo precorneal retention	HPLC [254]
	LC–MS/MS analysis [268, 274]
	Slit-lamp examination of fluorescence or Fluorescence imaging system using dye [191, 193, 195, 203, 208, 213, 250]
	SEM [261]
	Gamma scintigraphy[113, 179, 267]
In vivo ocular drug distribution/ocular bioavailability	Polarization fluoroimmunoanalysis [246]
	Radio labelled - liquid scintillation counter [118, 123, 248]
	HPLC [25, 178, 191, 193, 194, 249, 250, 263, 273]
	UV-Vis [261]
	single photon emission computed tomography image analysis [118]
	microdialysis [195]

Table 3

Chemical structures of synthetic polymers used for drug delivery to the anterior segment of the eye.

Name	Chemical Structures
Poly(alkyl-cyanoacrylate)	
Poly(lactide) (PLA)	
Poly(lactic-co-glycolic acid) (PLGA)	
Poly(ε-caprolactone) (PCL)	
Poly(ethylene glycol) (PEG)	
Poly(L-lysine)	
Carbopol®	

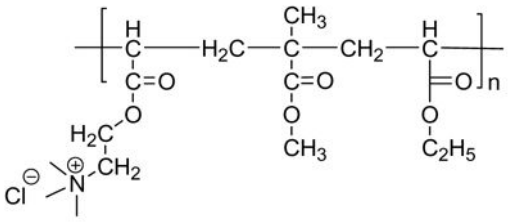
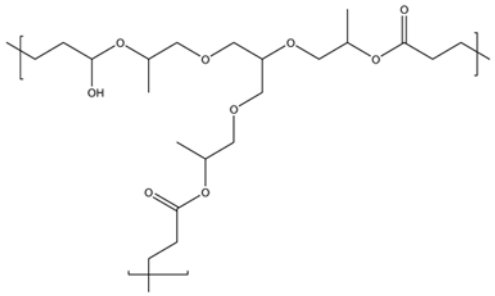
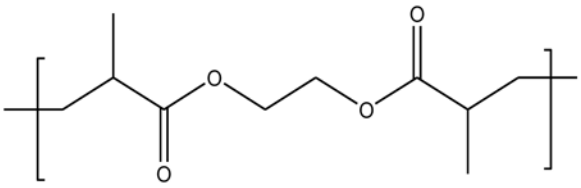
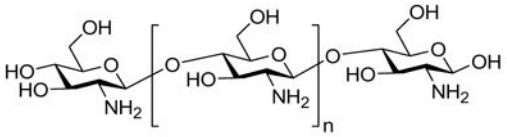
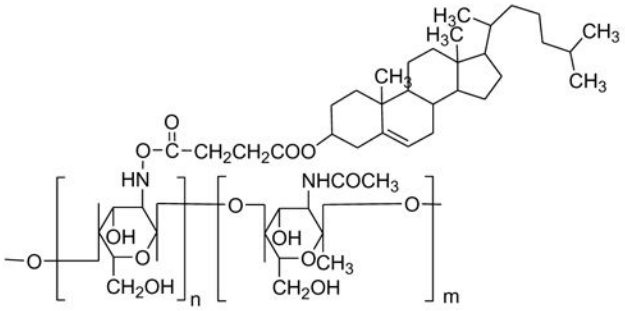
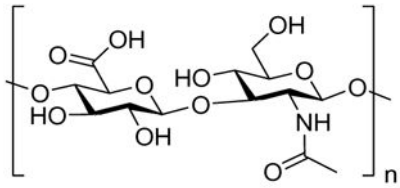
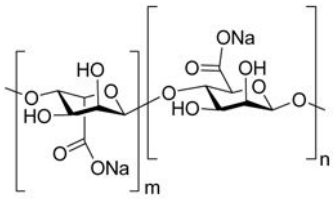
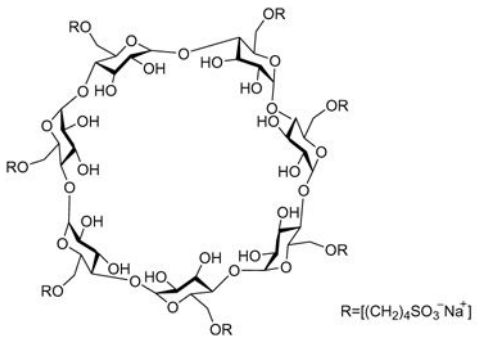
Name	Chemical Structures
Eudragit® RL	
Poly(propoxylated glyceryl triacrylate) (poly PGT)	
Poly(ethylene glycol dimethacrylate) (PEGDMA)	

Table 4

Chemical structures of polysaccharides used for drug delivery to the anterior segment of the eye.

Name	Chemical Structures
Chitosan	
Chlolesterol modified chitason (CS-CH)	
Hyaluronic acid (HA)	
Sodium alginate	
Sulfobutylether-cyclodextrin	

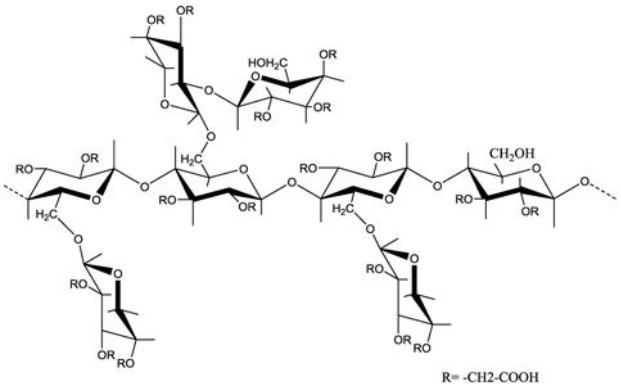
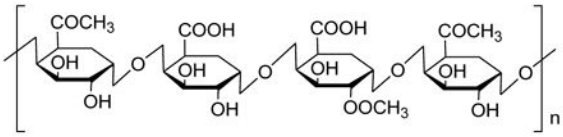
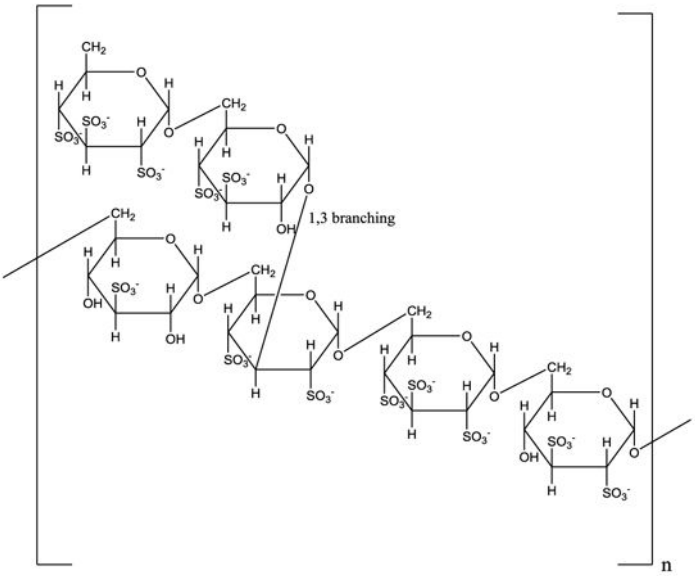
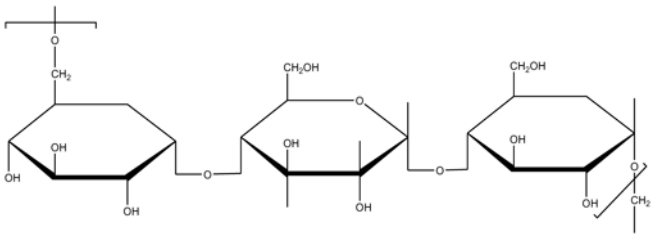
Name	Chemical Structures
Carboxymethyl tamarind kernel polysaccharide	
Pectin	
Dextran Sulfate	
Gum Cordia	Natural anionic gum (Structure N/A)
Pullan	

Table 5

Chemical structures of lipids used for drug delivery to the anterior segment of the eye.

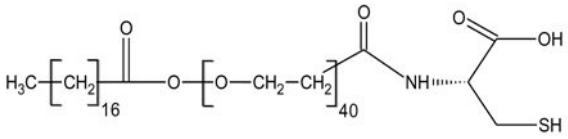
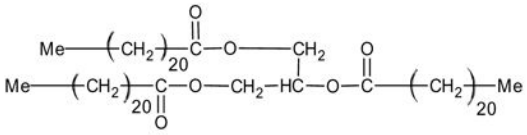
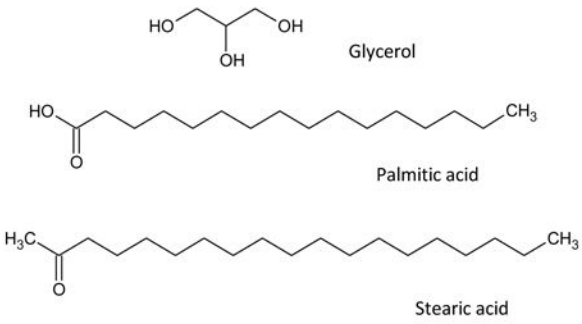
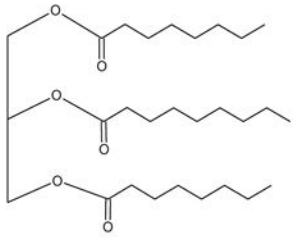
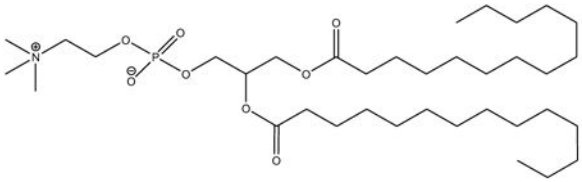
Name	Chemical Structures
Thiolated PEG stearate (cysteine-polyethylene glycol monostearate)	
Compritol 888 ATO (Behenoyl polyoxyl-8 glycerides NF) or Docosanoic acid,1,2,3-propanetriyl ester	
Precirol ATO 5 (Glycerol Palmito-Stearate)	
Gelucire 44/12 (Lauroyl polyoxyl-32 glycerides NF)	N/A
Miglyol 812	
Dimyristoyl phosphatidylcholine	

Table 6

Examples of drug-loaded PBCA nanoparticles.

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	*In Vitro* Release	*In Vivo* Observations	Ref
PBCA	pluronic F68	nanoprecipitation	pilocarpine	98±4	-	~10–60	10 h	nanoparticle-drug adsorbates showed prolonged myosis (rabbit)	[275]
PBCA	dextran 70k; dextran sulfate; n-acetylglucosamine	nanoprecipitation	betaxolol HCl	220 to 241	−0.5 to +12.5	~25–70	~40% in 2h	increased reduction in IOP (rabbit) compared to commercial drops	[276]
PBCA/poly(ethylene glycol) coating	pluronic F68; hydroxypropyl-b-cyclodextrin;	emulsion polymerization	acyclovir	190 to 450	−12.2 to −25.9	-	~40–65% in 15 min; 60–80% in 7 h	25-fold higher $AUC_{(0–360\ min)}$ compared to free drug suspension (rabbit)	[69]
PBCA	dextran 70k; Synperonic F68; sodium lauryl sulfate	nanoprecipitation	amikacin sulfate	83.75 to 453.3	−0.26 to −2.75	-	~70% in 3 h	achieved at least 3-fold higher drug concentrations of drug in aqueous humor than the control solution (rabbit)	[68]

Table 7

Examples of drug-loaded chitosan nanoparticles.

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	*In Vitro* Release	*In Vivo* Observations	Ref
Chitosan	TPP	ionotropic gelation	CsA	293±9	+37±0.9	73.4	~65% in 1 h and ~70% in 24 h	achieved therapeutic drug levels in external ocular tissues and maintained for at least 48 h	[90]
Chitosan	TPP	ionotropic gelation	dorzolamide HCl; pramipexole HCl	275 to 452	-	-	~60–80% in 4 h (dorzo); ~70–100% in 24 h (prami)	-	[91]
Chitosan	TPP; sodium alginate (in situ gel)	ionotropic gelation	dorzolamide HCl	164±10.2	-	98.1	~50–60% in 8 h	gamma scintigraphy showed retention of formulation at the corneal surface for at least 2 h	[267]
Chitosan	TPP	ionotropic gelation	acyclovir	200±30	+36.7±1.5	56 to 80	~20% in 1 h and 90% in 24 h	-	[277]
Chitosan	TPP	ionotropic gelation	5-fluorouracil	114 to 192	+30±4	8 to 34	~20% in 30 min and ~60% in 8 h	3.5 fold higher $AUC_{(0–8h)}$ of drug in aqueous humor compared to drug solution	[94]
Chitosan	TPP	ionotropic gelation	carteolol	~100 to 250	-	~40 to 80	~30% in 1 h and 80% in 24 h	gamma scintigraphy showed good spread and retention of formulation at the corneal surface compared to drug solution and prolonged IOP reduction	[113]
Chitosan	TPP	ionotropic gelation	ketorolac tromethamine	108 to 257	16.6 to 22.9	34.9 to 73.48	~100% in 5–6 h	-	[114]

Table 8

Examples of drug-loaded chitosan-based hybrid nanoparticles.

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	*In vitro* Release	*In Vivo* Observations	Ref
Chitosan–sodium alginate	pluronic ® F-127	ionotropic gelation (modified coacervation method)	gatifloxacin	205 to 572	+17.6 to +47.8 mV	77 to 82	90% in 24 h	na	[95]
Chitosan-sodium alginate	Tween® 80	ionotropic gelation	betamethasone sodium phosphate	16.8 to 692	+18.49 to +29.83	64	~95% release in 24–72 h	showed sustained release pattern *in vivo* in vitreous humor (at least 24 h) and good permeability of drug compared to the solution formulations	[96]
Thiolated chitosan (TCS)-alginate (ALG)	-	ionotropic gelation	FITC	265.7±7. 4 (CS-ALG) and 408.0±6. 4 (TCS-ALG)	+29.5±4. 1 (CS-ALG) +49.2±2. 3 (TCS-ALG)	84.2±1.8 (CS-ALG) 92.1±1.9 (TCS-ALG)	-	-	[278]
Hyaluronic acid-chitosan	TPP	ionotropic gelation	gene (plasmid PEGFP or PB-gal)	100 to 235	−30 to + 28	~99.9	-	provided high transfection levels (up to 15% of the cells);nanoparticles were internalized by fluid endocytosis, mediated by hyaluronic receptor CD44	[97]
Hyaluronic acid modified chitosan (CS-HA)	TPP-for unmodified	ionotropic gelation	timolol maleate (TM) / dorzolamide hydrochloride (DH)	143.9 ± 6.3 (chitosan) 319.5 ± 4.0 (CS-HA)	+34.1 ± 6.4 (chitosan) +33.3 ± 6.1 (CS-HA)	Chitosan: DH (91.0±2.9) and TM (54.2±1.0); CS-HA: DH(85.1±2.3) and TM(19.3±2.0)	~100% in 24 h (Chitosan); ~20% in 24 h (CS-HA)	in terms of overall reduction of ΔIOP *in vivo*, CS-HA nanoparticles outperformed chitosan nanoparticles and marketed formulation	[279]
Cholesterol modified chitosan (CS-CH)	-	self-aggregation through sonication or diafiltration	CsA	185.2 to 226.1	+43.14 to + 44.88	41.8	60% in 4 h and 95% in 48 h	showed good retention ability at the procorneal area while no activity was detected in the posterior segment	[118]
Cholesterol modified chitosan (CS-CH)-PLA	-	nanoprecipitation	rapamycin	312 to 326	+ 30.3	75.2 to 89.3	~25% in 4 h and ~85% in 8 days	chitosan-CH/PLA nanoparticles improved the immunosuppressive effect of rapamycin in corneal transplantation in rabbits as effectively as and even slightly better than rapamycin suspension in terms of	[253]

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	*In vitro* Release	*In Vivo* Observations	Ref
								the medial survival time of the corneal allografts (27.2 ± 1.03 days vs. 23.7 ± 3.20 days)	
Chitosan/Carbopol	-	ionotropic gelation	pilocarpine	294±30	+55.78± 3.41	77±4	~30% in 2 h and 65% in 24 h	during *in vivo* miotic study, the nanoparticle formulation showed four fold higher $AUC_{(0–24h)}$ compared to drug solution in terms of decrease in pupil diameter	[98]
Chitosan/sulfobutylether-beta-cyclodextrin	-	ionotropic gelation	econazole nitrate	90 to 673	+ 22 to 33	13.37 to 45.67	~50% in 8 h	nanoparticles provided greater antifungal effect to the eye surface than drug solution (rabbit)	[99]
Chitosan/sulfobutylether-beta-cyclodextrin	-	ionotropic gelation	naringenin	446.4 ± 112.8	+22.5 ± 4.91	67.10 ± 0.26%.	~100% in 5 h	increased $AUC_{(0-t)}$ (4.6-fold) and C_{max} (3.7-fold) of the drug compared to drug suspension	[280]
lecithin/chitosan	-	ionotropic gelation	natamycin	213.83 ± 2.02	43.83±1. 80	73.57±0.62	~40% in 2 h and ~60% in 7 h	increased (~1.5 fold) $AUC_{(0-inf)}$ of natamycin in precorneal region compared to drug solution; total drug available above the *MIC* (*AUC* above *MIC*) by nanoparticles was ~1.53 fold higher than drug solution	[100]
Chitosan - alginate	-	ionotropic gelation	daptomycin	~375 to 470	−20 to +25	~60 to 99	-	-	[89]
Lecithin-chitosan	-	ionotropic gelation	amphotericin B	161.9 to 230.5	+26.6 to +38.3	70 to 75	~80% in 10h	improved drug levels (~2.04 fold) and precorneal residence time (~3.36 fold) of nanoparticles compared to marketed formulation	[101]
chitosan or alginate nanoparticles	polaxamer 188; lecithin; PVA; HPMC; Methocel; Triton^{TM} X-100	spontaneous emulsification/solvent diffusion	celecoxib	113.33± 4.08 (chitosan); 154.67± 5.06 (alginate)	+36.92± 3.38 (chitosan) - 36.5±4.7 (alginate)	89.88±4.17 (chitosan); 75.38±2.98 (alginate)	after 24 h, drug release from CS-NPs & ALG NPs preparations eye drops, preformed gel and the in situ gelling system	-	[102]

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	*In vitro* Release	*In Vivo* Observations	Ref
							was (61.5, 50.1, 44.6%) and (51.3, 46.7 and 40.1%) respectively.		
hyaluronic acid coated chitosan	TPP	ionotropic-gelation	dexamethasone	~305 (before coating); 400 (after coating)	+32.55 (before coating); −33.74 (after coating)	72.95	~30% in 1 h and ~65% in 12 h	chitosan nanoparticles and HA-coated chitosan nanoparticles showed 1.83-fold and 2.14-fold higher $AUC_{(0-24\ h)}$ in rabbit aqueous humor, respectively compared to dexamethasone solution	[271, 273]

Table 9

Examples of drug-loaded polysaccharide-based nanoparticles (other than chitosan).

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	*In Vitro* Release	*In Vivo*	Ref
Thiolated pectin	magnesium chloride	ionotropic-gelation	timolol maleate	237	-	94.6	-	-	[104]
Sodium hyaluronate; Zinc hyaluronate	-	carbodiimide technique for crosslinked structures	sodium diclofenac	<110	-	100	showed an initial burst release of model drug sodium diclofenac at ~35–55% in 1 h, and released up to ~60–88% at 6 h	-	[106]
Gum cordia	polyvinyl alcohol/calcium chloride	emulsion cross-linking technique	fluconazole	315 to 394	−55 to −48	65 to 93	-	-	[105]
Tamarind seed xyloglucan nanoaggregates	poloxamer-407	nanoaggregation	tropicamide	639.8	-	96.57	-	-	[258]

Table 10

Examples of drug-loaded polyester-based nanoparticles.

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	*In Vitro* Release	*In Vivo* Observations	Ref
PLGA	poloxamer 188	Solvent displacement	flubiprofen	~ 200 to 300	−25.3 to −26.1	~85 to 95	100% in 8 h	-	[134]
PLGA	PVA	nanoprecipitation	sparfloxacin	~ 180 to 190	−22.5 ± 1.8	~25 to 86	~80% in 24 h	radiolabeled sparfloxacin-loaded PLGA nanoparticles were cleared at a much slower rate and retained for a much longer duration at the corneal surface than commercial sparfloxacin eye drops.	[137]
PLGA; poly (lactide-co-glycolide-leucine)	Tween® 80	o/w emulsion/solvent evaporation	diclofenac sodium	~125 to 174 [poly(Lac(Glc-Leu)] 160 to 190 (PLGA)	~ −25 mV for both	~28 to 95 (PLGA); ~17 to 73 (PLGL)	~50% in first 2 h follow ed by a slow-release for up to 14 h (90%)	-	[142]
PCL (chitosan or poly-L-lysine coating)	lecithin; Miglitol® 840	interfacial deposition technique	indomethacin	238±6 (uncoated); 251±5 (PLL coated); 384±60 (CS coated)	−39.9±0. 3 (uncoated); +27.9±3. 5 (PLL coated); +37.1±l. 8 (CS coated)	94.5±4.3 (uncoated); 93.1±1.7 (PLL coated); 91.8±8.9 (CS coated)	~95% in 4 h (both)	uncoated and PLL coated PCL nanocapsules increased the indomethacin concentrations in the cornea (~3.2 fold increase in *AUC*) and aqueous humor (~4.3 fold increase in *AUC*) than commercial eye drop Indocollyre after topical instillation while CS coated PCL showed ~5.4 fold in *AUC* in cornea and 6.7 fold in *AUC* in Aqueous humor.	[248]
PCL; poly-D-glucosamine (PDG)-PCL	pluronic® F-68 and	nano-precipitate/sol vent displacement	natamycin	178.8±1.70 (PCL); 217.0±2.00 (PDG-PCL)	−21.3±1.23 (PCL); +43.5±1. 55 (PDG-PCL)	61.83±0.07 (PCL); 77.85±1.08(PDG-PCL)	~100% in 8 h (both)	PDG-PCL NPs showed ~6 fold increase in *AUC* precorneal region and ~6.3 fold decease in clearance compared to commercial drug suspension	[138]
PLA; PLA; PLGA	lecithin; PVA or poloxamer 188	spontaneous emulsification solvent diffusion	brimonidine	117.33±4.58 (PCL); 131.67±3.79 (PLA); 125.67±5.15 (PLGA)	−18.50±2. 87 (PCL); −28.11±2. 21 (PLA); −21.82±2. 7 (PLGA)	77.97±1.38 (PCL); 73.52±2.92 (PLA); 68.65±3.35 (PLGA)	~60% in 24 h (PCL); ~50% in 24 h (PLA); ~54% in 24 h (PLGA)	a single topical dose of the nanoparticles maintained a reduced IOP for greater than 16 h.	[260]
PCL	Migliol® 840		CsA	210 to 270	−55 to −60	>99%	~75 in 5 h	showed enchanced conreal penetration after topical insitalation -5 fold higher than oily solution of drug.	
PCL/Benzalkonium chloride – HA coating	-	nanoprecipitation	CsA	200 to 300	Positive zeta	-	-	nanoformulations achieved high levels (~10–15 fold higher) of CsA in the cornea than that is achieved with castor oil solution of CsA.	[147]

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	*In Vitro* Release	*In Vivo* Observations	Ref
phenylboronic acid surface functionalized poly(D, L-lactide)-b-dextran	-	nanoprecipitation	CsA	35.6±7.4	−31.3±1.5	~ 40	~90% in 120 h	“once a week administration of CsA loaded nanoparticles achieved both the elimination of inflammatory infiltrates and recovery of the ocular surface, whereas thrice a day administration of commercial product Restasis® (Cyclosporine Ophthalmic Emulsion, 0.05%) only showed clearance of the inflammatory processes”	[150]

Table 11

Examples of drug-loaded Eudragit-based nanoparticles.

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	*In Vitro* Release	*In Vivo* Observations	Ref
Eudragit ® RS100	Tween® 80	quasi-emulsion solvent diffusion	ibuprofen	40 to 110 nm	+40 to +60		100% in 24 h	nanosuspension showed enhanced drug levels (1.6 fold higher) in aqueous humor compared to drug solution	[249]
Eudragit ® RS100/RL 100	PVA	w/o/w emulsification solvent evaporation and high pressure homoginization	amikacin	~ 120 to 250	+25 to +46.3	72.16 to 80.94	~40 to 90% in 12 hr (depending on formulation)	showed corneal adhesion for at least 6 h; enhanced *AUC* drug levels (~21.fold) compared to the drug solution	[163]
Eudragit ® RS100/RL 100 Coated with HA	-	spontaneous emulsification technique	Gatifloxacin/prednisolone	315.2 to 973.65	+30 to +45	70 to 86 (gatifloxacin); 61 to 79 (prednisolone)	72 to 85% in 24 h (gatifloxacin); 40–43% in 24 h (prednisolone)	enhanced corneal drug levels compared to solution (C_{max}: ~5.2 fold/$AUC_{(0–6h)}$: ~9.3 fold); Enhanced aqueous humor drug levels compared to solution (C_{max}: ~1.8 fold/$AUC_{(0–6h)}$: ~1.8 fold);	[156]

Table 12

Examples of drug-loaded lipid based nanoparticles.

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	*In Vitro* Release	*In Vivo* Observations	Ref
Epikuron® 200 (soya phosphatidylcholine 95%)	stearic acid; sodium taurocholate	o/w microemulsion; diaultrafiltration	tobramycin	70 to 80	-	-	-	enhanced *AUC* drug levels (~4 fold) compared to drug solution	[172]
Softisan® 100	stearic acid or palmitic acid; Tween® 80	quasi-emulsion solvent diffusion	melatonin	175 to 303	+58 to +59	90 to 95	~ 60% in 8h	caused significant IOP lowering in albino rabbits for about 24 h	[177]
Dynasan® 116 (triplamitin)	Glyeryl dibehenate; Tween 80; Octadecylamine	hot homogenization	CsA	248±0.33	50.30±0.78	-		increased residence time in eyes and released the *in vivo* drug for 48 h	[176]
Compritol® 888 ATO	poloxamer 188; Tween 80	high shear homogenization and ultrasound method	CsA	225.9±5.5	−16.9±0.7	95.6	-	CsA could penetrate into the aqueous humor at therapeutic level two hours after topical instillation of CsA loaded SLN into rabbit eyes	[173, 174]
Compritol® ATO 888; Miglyol® 812	cremphor® EL 40; Gelucire® 44/14; stearic acid; transcutol® P	melted-ultrasonic method	ibuprofen	69	+28.9	95	-	showed increase AUC drug levels (4-fold) in aqueous humor compared to control	[182]
Cysteine-polyethylene glycol monostearate (thiolated)	Precifac® ATO 5; 30% Miglyol® 840; Tween® 80	emulsification-probe sonicator	CsA	68	-	-	~70% in 48 h	showed increase *AUC* drug levels (~4.7-fold chitosan modified; ~2.2-fold unmodified) in aqueous humor compared to drug solution	[189]
Soy lecithin; Chitosan Coating	Glycerol; Pluronic® F68; soyabean oil	Film-dispersion high-pressure homogenization system	Dexamethasone	155.03±1. 47 (chitosan coated) 132.25±0. 07 (uncoated)	+29.99±4.63 (coated) −25.73±0.78 mV (uncoated)			Showed increased *AUC* drug levels (4 fold) in aqueous humor compared to control	[194]
Miglyol® 812 N; Glyceryl Monostearate; N-acetyl-L-cysteine functionalized chitosan copolymer (CS-NAC) (surface modification)	Solutol® HS15; Gelucire® 44/14	melt emulsification technique.	curcumin	50.76 ± 2.21 (unmodified); 88.64 ± 1.25 (modified)	−20.38 ± 0.39 (unmodified); 22.51 ± 0.34 (modified)	>95	~15% in 10 h and ~ 32% in 72 h	Coating of NLC with CS-NAC resulted in sustained release, increased precroneal retention and enhanced bioavailability of curcumin.	[191]
Glycerin mono stearate and Miglyol® 812; QACMC modification	Gelucire® 44/14; Tween® 80	film-ultrasonic technique.	curcumin	158.1	+36.5	99.12	(~60–80% in 4 h; ~80–90% in 12 h)	Fluorescene imgaing showed prolonged retention of the nanoparticles, and in comparision to the circumin eye drops, C_{max}, $AUC_{0-\infty}$, $MRT_{0-\infty}$ of the formulations unmodified-NLC (1.2-, 1.5-, and 1.8-fold), cationic-modified NLC (1.3-,2.4-, and 2.3-fold) were significantly higher.	[191].
Compritol® Palmitic Acid	Poloxamer 188; soya lecitihin; sodium taurocholate	solvent emulsification/evaporation method.	voriconazole	139–334	>−30	40–64	~20–40% in 2 h and ~60–80% in 12 h	*In vivo* pharmacokinetic parameters $AUC_{(0-12\ h)}$ (2-fold), *MRT* (1.2-fold) of voriconazole were significantly higher than the plain drug suspension	[178].

Primary Polymer	Other Component	Fabrication Method	Drug Encapsulated	Size (nm)	Zeta Potential (mV)	Encapsulation Efficiency (%)	*In Vitro* Release	*In Vivo* Observations	Ref
Glyceryl monostearate	PVA; Tween® 80k Pluronic® F-68	melt-emulsion sonication and low temperature-solidification process	celecoxib	198.77±7. 5	−10.6±0.8 to −16.2±0.6	65.42 to 93.91	~70% in 8 h	Results from the gamma scintigraphy using ^{99m}Tc labeled SLNs demonstrated that the nanoparticulate systems showed prolonged retention over ocular surfaces in vivo in rabbits	[179]
Glycerin monoolein	Ploxamer 407; gelucire® 44/14; octadecyl-quaternized carboxymethyl chitosan	sub-sequent evaporation; high shear	tetrandrine	170.0 ± 13.34	29.3± 1.25	95.46 ± 4.13 %,	~70% in 2 h and 90% in 11 h	In vivo rabbit aqueous pharmacokinetic parameters $AUC_{(0-5\ h)}$ (2-fold) of tetrandrine were significantly higher than drug solution	[195]
Compritol® 888 ATO; PEG 600	Tween® 80	High pressure homogenization	ketoconazole	126.35±2. 2	−3.19	70.19±1.94		Showed 2.5 fold higher bioavailability (*AUC*) in rabbit aqueous humor	[30]

Table 13

Examples of drug-loaded dendrimers.

Primary Polymer/s	Drug Encapsulated	Size (nm)	Encapsulation Efficiency (%)	*In Vitro* Release	*In Vivo* Observations	Ref
poly(amidoamine) (PAMAM) dendrimers	pilocarpine nitrate and tropicamide	-	-	-	PAMAM dendrimers with carboxyl or hydroxyl terminal surface groups increased the miotic effect of pilocarpine nitrate with 1.39–1.64 fold increase in *AUC* and the mydriatic activity of tropicamide with 1.13–1.34 fold increase in *AUC* in comparison with 1% drug in phosphate buffer	[203]
phosphorus-containing dendrimers having a quaternary ammonium as core	carteolol	-	-	-	G2 dendrimers caused a 2.5 times increase in the concentration of carteolol in the aqueous humor in comparison with the drug suspension alone	[204]
dendritic polyguanidilyated translocators	gatifloxacin	346	-	~ 60 % in 3 h and ~ 70% in 4 h	in comparison with the drug solution alone, the nanoparticles increased the drug levels in the cornea and conjunctiva by ~2 and ~13 fold, respectively; and prolonged the retention time in the aqueous humor from 2 to 24 h	[207]
poly(propyleneimine) dendrimers	acetazolamide	40 to 60	56±2.3	~83% in 24 h	in comparison with acetazolamide solution alone, the dendrimer formulation increased the corneal residence time of acetazolamide by ~5–7 fold and prolonged the IOP-lowering effect of acetazolamide from 2 h to ~4 h	[208]

Table 14

Examples of drug-loaded nanoparticle-based composite system.

Composite System	Hydrogel material	Primary Polymer of nanoparticle	NP Fabrication Method	Drug Encapsulated	Size (nm)	Encapsulation Efficiency (%)	*In Vitro* Release	*In Vivo* Observations	Ref
Liposome in Contact lens	pHEMA	dimyristoyl phosphatidylcholine (DMPC)	lipid film hydration	levofloxacin	20	-	sustained *in vitro* release of lidocaine with 65% drug release in 7 days and an initial burst of about 15 to 30%	-	[210]
Liposome in contact lens	pHEMA	soya lecithin, cholesterol	lipid film hydration	ciprofloxacin	338.32 (MLV); 120.42 (ULV)	42 to 59	40 to 60% in 8h	contact lens coated with ciprofloxacin-entrapped liposomes showed much longer *in vitro* residence time than ciprofloxacin eye drops (24 h vs. 2–5 min)	[219]
Liposome in *in situ* gel	deacetylated gellan	soybean phosphatidyl-choline and cholesterol (0.3	pH-gradients method coupled with reverse evaporation	timolol maleate	136±18	47.3 to 83.31	composite system showed more sustained *in vitro* timolol maleate release than liposome nanoparticles alone (24 vs. 2.5 h for ~90% release).	composite system increased timolol maleate's corneal retention time (>10 min retention time vs. clear by 10 min) and IOP-lowering capability (~5 h vs. ~3 h duration of action) in rabbits more than timolol maleate eye drops	[213]
Core-shell nanospheres in contact lens	pHEMA	Pullan-PCL copolymer	micelle formation	ciprofloxacin	142±12	-	-	-	[231]
Surfactant-laden contact lens	pHEMA	Brij 97, Brij 98, Brij 78 and Brij 700	polymerizing the monomer solution	CsA	-	-	> 10 to 50 days depending on surfactant	-	[221, 222]
Polymeric nanoparticle in *in situ* gel	carbopol 934P	chitosan	ionic gelation	norfloxacin	158.4 to 345.8	64.77 to 77.38	~80% in 12 h (composite system)	-	[281]
Polymeric nanoparticle in contact lens	pHEMA	poly(ethylene glycol dimethacrylate) (poly EGDMA), poly (propoxylated glyceryl triacylate) (poly PGT)	emulsion polymerization	timolol	3.5	48 to 66	composite system showed increased drug release duration from 1 to 2 h to about 2 to 4 weeks	-	[211]
Polymeric nanoparticle in contact lens	poly-silicone	(poly PGT)	emulsion polymerization	timolol	3.5	-	-	preliminary *in vivo* studies in beagle dogs demonstrated safety and efficacy of this composite system in lowering IOP for 4 days	[217]
Polymeric nanoparticles in contact lens	pHEMA	chitosan	Ionotropic gelation	dexamethasone	131 – 170	~21 to 43	~ 50% in 23 days (composite system)	-	[228]
Polymeric nanoparticles in contact lens	pHEMA	pEGDMA	free radical solution polymerization	lidocaine	20–50	-	~100% in 9 days (composite system)	-	[282]

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Composite System	Hydrogel material	Primary Polymer of nanoparticle	NP Fabrication Method	Drug Encapsulated	Size (nm)	Encapsulation Efficiency (%)	*In Vitro* Release	*In Vivo* Observations	Ref
Polymeric nanoparticles in contact lens	Poly(HEMA -co-N-vinylpyrrolidone); PEG-diacrylate as cross linker	PCL-HEMA-PEG-diacrylate	miniemulsion polymerization	loteprednol etabonate	52.3 to 83.4	60.3 to 71.4	~55% in 12 d (composite system); ~50% in 4 days (nanoparticles alone); ~55% in 2 days (contact lens alone)	‘	[283]