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Review article

Polymer-based carriers for ophthalmic drug delivery

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ABSTRACT

Despite the wide range of diseases affecting the eye, ocular bioavailability remains a challenge in ophthalmic drug delivery. Nowadays an extensive variety of polymers are being explored to develop colloidal drug carriers which show better performance than the more popular drug solutions. For instance, regardless of the type of polymer used, these systems prolong the residence time of the drug in the absorption site with respect to conventional aqueous eye drops which are rapidly cleared from eye surface. Furthermore, colloidal drug carriers can be internalized by cells. In addition, positively charged particles penetrate the cornea more effectively than neutral or negatively charged ones. These phenomena lead to higher ocular bioavailability. This review overviews the different polymers available to produce drug-loaded gels, microparticles and nanoparticles, highlighting the advantageous features and biocompatibility of each polymer and the major achievements in the field of ocular delivery. In addition, the design of more complex delivery systems that combine several delivery platforms is presented. Finally, regulatory aspects relevant to the clinical translation of advanced ophthalmic drug delivery systems are also discussed. All together, this manuscript is aimed at guiding pharmaceutical research and development towards the rationale polymer selection to produce drug delivery systems that improve the performance of drugs for the therapy of ophthalmic diseases.

1. Introduction

1.1. Anatomy of the eye and administration approaches in ophthalmic delivery

The eyeball is divided into the anterior and posterior area (Fig. 1). The first one includes the cornea, the crystalline lens, the iris, the ciliary body and the fluid-filled aqueous humor, while the second comprises the sclera, choroid vessels, the retina, the macula, the optic nerve, and the jellylike fluid known as vitreous humor.

Topical administration (e.g., eye drops) is the most attractive one owing to ease of application, more limited side effects than systemic administration and high patient comfortability and compliance, and it is relatively efficacious to treat diseases of the anterior eye. The main drawback is the short residence time of liquid formulations. Conversely, to reach the posterior area, drugs need to cross the tear film, the mucus layer, the conjunctiva and the cornea that usually reduce absorption and limit bioavailability.

Tears constitute a fluid secreted by the lachrymal glands that cover the cornea, forming a film consisting of three layers: the outer oily one

Abbreviation: ABC, ATP-binding cassette superfamily; ANDA, Abbreviated new drug application; AUC, Area-under-the-curve; BSA, Bovine serum albumin; DDS, Drug delivery system; EPR, Enhanced permeability and retention; FITC, Fluorescein isothiocyanate; G, α-L-guluronic acid; GA, Glycolide; GRAS, Generally Recognized as Safe; HCEC, Human corneal epithelial cells; HLEC, Human lens epithelial cells; HET-CAM, Hen's Egg Test Chorioallantoic Membrane; HSA, Human serum albumin; IOP, Intraocular pressure; LA, Lactide; M, β-D mannuronic acid; MIC, Micelles; MP, Microparticle; MRP, Multidrug resistance-associated protein; MRT, Mean residence time; MTT, Methyl thiazol tetrazolium; NDA, New drug application; NIMDS, Nanoparticle-in-microparticle delivery system; NP, Nanoparticle; OVA, Ovalbumin; ODDS, Ophthalmic drug delivery system; PACA, Poly(alkyl cyanoacrylate); PCL, Poly(epsilon-caprolactone); PDLA, Poly(D-lactide); PDLLA, Poly(DL-lactide); PEG, Poly(ethylene glycol); pEGFP, Enhanced green fluorescent protein plasmid; PEO-PPO-PEO, Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide); PGE2, Prostaglandin E2; P-gp, P-glycoprotein; PGA, Poly(glycolide); PLA, Poly(lactic acid); PLGA, Poly(lactic-*co*-glycolic acid); PLLA, Poly(L-lactide); PNiPAAm, Poly(*N*-isorpropyl acrylamide); POE, Poly(orthoester); RPEC, Retinal pigment epithelial cells; RVEC, Retinal vascular endothelial cells; siRNA, Small interfering RNA; TPP, Tripolyphosphate; TEER, Transepithelial electrical resistance; TUNEL, Terminal deoxynucleotidyl transferase-mediated dUTP nick end; US-FDA, US-Food and Drug Administration; UV, Ultraviolet; VEGF, Vascular endothelial growth factor

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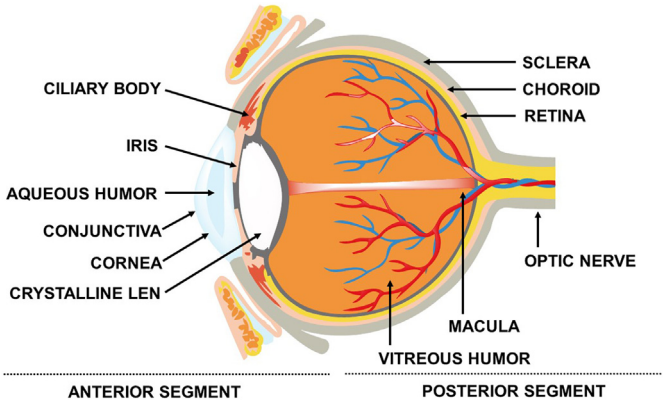

Fig. 1. Structure of the human eye.

minimizes the evaporation of water and ensures appropriate lubrication, the middle layer is aqueous and contains the antimicrobial enzyme lysozyme (also known as muramidase or *N*-acetylmuramide glycanhydrolase) and the inner one is rich in glycoproteins such as mucin [1–3]. After topical administration, an excess of tears is produced by the lacrimal glands that wash the ocular surface, draining the drug by the nasolacrimal sac towards the systemic circulation and eventually leading to systemic side effects. Hence, lachrymation is associated with short residence time in the eye (1-2 min). Then, the remaining drug has to cross the dense conjunctival tissue, a vascularized mucus tissue that covers the inner surfaces of the eyelids and the eyeball to protect them [4]. Owing to its vascularization most of the drug that enters the conjunctiva is absorbed into the systemic circulation, being unable to reach the inner parts of the eye to elicit its pharmacological activity. Finally, the drug faces the cornea that represents an anatomical barrier formed by three primary layers: the epithelium (adjacent to the conjunctiva), the stroma (the middle layer) and the endothelium (the inner one). The epithelium and the endothelium are rich in lipids limiting the absorption of hydrophilic drugs, while the stroma is very rich in water and restricts the permeability of lipophilic ones. In addition, the cornea can detect pH and osmolality changes and induce reflex blinking and tearing further contributing to drug clearance. Moreover, both conjunctiva and corneal epithelium present tight junctions that constrain the absorption of any substance (regardless of its physicochemical properties) *via* paracellular permeation jeopardizing even more the delivery [5]. Another mechanism that challenges ocular delivery is the presence of efflux pumps (e.g., ATP-binding cassette superfamily) that are widely distributed in the conjunctiva and cornea [6]. All these barriers reduce the bioavailability in the cornea to less than 5%. Considering that the volume of commercial eye drops is approximately 30 μL which is the volume of the conjunctival sac in humans and that, after a single blink, only approximately 10 μL remains, the drug amount that is dispersed/solubilized in this volume is extremely low [7]. This imparts the need of frequent administration regimens to achieve the therapeutic effect, compromising patient compliance. Furthermore, this poor bioavailability constrains the use of topical administration to treat disorders of the anterior eye, while other administration routes are demanded to reach the posterior segment. Among them, systemic administration is one option although the fraction of drug that reaches the retina is very small due to the presence of the blood-retinal barrier that is formed by outer retinal pigment epithelium cells and an inner retinal endothelium. In addition, cells present tight junctions [8] and several ABC transporters, mainly P-gp, MRP1, MRP4 and MRP5, are expressed at the outer and inner layer [9–11]. These mechanisms limit drug absorption from the bloodstream into the retina. Therefore, large systemic doses are required to achieve therapeutic drug concentrations in the retina, leading to off-target toxicity. To improve drug bioavailability in the posterior segment of the eye, periocular injections including the subconjunctival, subtenon, juxtacleral, peribulbal and retrobulbar are used [12]. These injections interface the drug near the sclera, which allows the diffusion of the drug into the retina and the vitreous (Fig. 1). In this case, the blood-retinal barrier also hinders drug absorption. Hence, intravitreal injections are needed to deliver drugs directly to the vitreo-retinal region and achieving therapeutic drug levels in the action site; e.g., bevacizumab (Avastin®) injections to treat diabetic macular edema or age-related macular degeneration [13,14]. However, many drugs have short intravitreal half-life (2-10 h) and demand frequent administration as well [15]. Moreover, it is an invasive route associated with adverse effects and low patient compliance.

Aiming to overcome these remarkable therapeutic challenges, over the last two decades, there was an explosive growth of the research in the field of ophthalmic drug delivery. In this context, polymers became fundamental players owing to outstanding design and synthetic versatility and modularity, the ability to produce drug delivery systems with a broad range of sizes, from micrometers to nanometers, shapes and properties (e.g., mucoadhesiveness) [16]. In this review, we overview in a very comprehensive manner the polymers used to produce ophthalmic drug delivery systems (ODDS) for the treatment of ocular diseases, with special emphasis on microparticles (MPs) and nanoparticles (NPs). Also, the most attractive vehicles to administer the ODDS are detailed. The most advantageous features and biocompatibility of each polymer together with the major achievements in the field of ocular delivery are presented. In addition, the design of more complex delivery systems that combine several delivery platforms is analyzed. Finally, regulatory aspects relevant to the clinical translation of advanced ophthalmic drug delivery systems are also discussed.

1.2. Requirements of an ophthalmic formulation

Ophthalmic formulations must meet certain general requirements to ensure maximum comfort to the eye and minimize local irritation, lachrymation and blinking, the latter increasing drug loss. Moreover, ODDS must be sterile to prevent the development of eye infections. These properties condition the physicochemical and mechanical properties of the polymers used in the design of ODDS. The optimum pH of an ocular formulation is 7.2 ± 0.2. However, the buffering capacity of the tears allows to tolerate pH values in the 3.5-8.5 range. Other parameters to consider are refractive index, surface tension and viscosity. These properties must be as close as possible to the physiological conditions (Table 1) to minimize the influence of the normal behavior of the tears and the pseudoplastic precorneal film [17,18]. Formulations with slightly higher viscosity prolong the residence time on the precorneal tissue, improving bioavailability. Conversely, too viscous systems may compromise the ocular comfort and even a proper instillation of the dosage form [19].

In the case of particulate ODDS for topical administration, the size should not exceed 10 μm to avoid ocular disturbance [20] and particles must be administered in a maximum volume of 30 μL which is the one that the human conjunctival sac can retain without blinking [21]. Conversely, for the intravitreal route, the particle size and the refractive index should be adjusted to reduce light scattering and ensure proper vision. The larger the particle size, the lower the particle concentration tolerated. This has a direct impact on the maximum dose that can be

Table 1
General requirements that must be fulfilled by an ocular formulation.

Parameter	Value
pH	6.5–7.6
Refractive index	1.35 ± 0.01
Superficial tension (mN m^{-1})	40–50
Viscosity (mPa.s)	~20
Osmolarity (mosmol/kg)	220–450

injected [15]. The utmost volume in which particles can be administered into the vitreous is 100 μL to avoid reflux concerns and blockage of aqueous humor circulation, whereas the sub-conjunctival route tolerates up to 500-1000 μL. In addition to these parameters, the carrier-forming biomaterial must comply with safety requirements. This issue will be thoroughly discussed in the following section. Regarding sterility, it is convenient to perform a final sterilization step rather than an aseptic production process. If the ODDS has a size below to 200 nm, it is possible to conduct sterile filtration, a process that does not compromise the physicochemical characteristics of the delivery system. Larger particles must be sterilized by autoclaving, irradiation or treatment with ethylene oxide and gas plasma. Autoclaving often generates aggregation of particles and changes in their biological activities [22]. In addition, the chemical and thermal stability of the polymer has to be ensured. Ethylene oxide is less recommended due to the toxicity and carcinogenicity of its residues. Conversely, gas plasma has the advantage of being non-toxic and using low temperature. Notwithstanding, since the mechanism of action is related to oxidation and reduction effects on microbial structures, it can compromise the functional groups of the ODDS-forming polymer and eventually of the active cargo [22]. Finally, the most widely used sterilization method is irradiation because it does not utilize neither heat nor chemicals, avoiding toxic residues after sterilization. Irradiation can be performed either with γ radiation, electronbeam or X-rays. However, the performance has to be validated for each single case because high doses of radiation can lead to physicochemical changes in the polymers and/or the ODDS and the drug [22].

2. Polymers for the production of particulate ophthalmic drug delivery systems

There exists a wide range of polymers that comply with the conditions for ophthalmic administration. They can be classified into natural, semisynthetic (chemically modified natural polymers) and synthetic polymers according to their origin. Although most of them are biodegradable, meaning that they possess enzymatically or hydrolytically cleavable chemical bonds, non-biodegradable prototypes like Eudragit®, a family of poly(methacrylate)s, emerged as a promising alternative as well [23–25]. As we will discuss below, each polymer has specific advantages and the choice needs to be done based on the administration site and the required residence time. For example, some of them are mucoadhesive and interact with the cornea and conjunctiva, prolonging the residence time of the encapsulated drug and enhancing drug absorption and bioavailability [26,27], while others enable a more controlled release of the cargo that reduces the frequency of administration [28]. Furthermore, polymers usually display functional groups in the side-chain that enable the grafting of ligands to target specific cells [29-31]. It is also worth pointing out that beyond the advantageous features of each polymer, particulate ODDS display better performance than the corresponding polymeric solutions. For instance, regardless of the mucoadhesive properties of the polymer, particulate material can be retained in the ocular mucosa for longer times than solutions [32]. Furthermore, in the case of leaky vasculature in tumors or inflamed areas NPs can be retained to a greater extent than in healthy tissues, improving passive drug targeting [33–35]. Furthermore, small particles can be endocytosed, increasing drug retention and penetration; e.g., usually, the smaller the size, the greater the cellular uptake, though other features such as surface properties and shape also govern these pathways [36]. Additionally, these systems can protect the payload from chemical and enzymatic degradation, which is critical for peptide- and DNA-based drugs [37]. Finally, NPs and MPs have sizes that are well-tolerated by the topical route and do not produce blurred vision as some polymer solutions. Despite the foregoing advantages, the potential cell toxicity of the carrier must be always evaluated in the relevant cell type/s. This phenomenon can be related to the polymer and additives (physicochemical characteristics, purity and source), the platform produced (particle size, morphology and concentration), the production process, the type of target cell and the administration route. It is worth stressing that although tolerability, irritation and cell toxicity are usually studied for each polymeric particle, phenomena such as aggregation may dramatically change the interaction with the biological mileu. For example, the formation of particle aggregates causes discomfort and leads to blinking, mild opacity of the cornea, edema of the conjunctiva and swelling of lids.

In the following sections, the most relevant polymers used in the production of ODDS will be described, highlighting the main achievements in this field (Table 2) and emphasizing the ocular safety of these systems.

2.1. Natural polymers

2.1.1. Alginate

Alginate is a natural and anionic polysaccharide extracted from soil bacteria and brown algae (Phaeophyceae), specifically from *Laminaria hyperborea*, *Laminaria digitata*, *Laminaria japonica*, *Ascophyllum nodosum* and *Macrocystis pyrifera* [215]. Although, commercially available alginates are obtained exclusively from the last source. Chemically, alginate is a linear unbranched copolymer consisting of β-D mannuronic acid (M) and α-L-guluronic acid (G) which are arranged as homopolymers (M block or G block) or heteropolymers (MG block) (Fig. 2).

The ratio and order of these monomers in the backbone as well as the length of each block and the total molecular weight vary with the source and the season, obtaining polymers of molecular weight between 32 and up to 500 kg/mol and with different viscosity levels [215,216]. The higher the molecular weight, the greater the viscosity. This is critical to enable flow through a syringe needle [217]. Similarly, as the pH decreases below 4.5, the viscosity increases due to the protonation of carboxylate groups (pK_a ~3) and the formation of H bonds [215]. Multivalent cations (e.g., Ca^{2+}) can bind to the carboxylic groups of two adjacent guluronate residues through electrostatic interactions, resulting in a well-known ionotropic crosslinking (Fig. 2) [218]. If the crosslinking process is carried out by drop wise addition into a crosslinker solution, the result is a hydrogel bead or MP that can be used to encapsulate water-soluble [38,176] and hydrophobic drugs [40] where the diameter depends on the size of the original droplet; the use of nozzles of different diameters enables good control of the droplet size. As alginate beads and MPs are produced under mild pH and temperature conditions and without utilizing organic solvents, this polymer is ideal to encapsulate therapeutic proteins and nucleic acids [219]. The gelation rate is a key factor in controlling the gel uniformity and mechanical properties that are directly related to the drug release kinetics. The slower the gelation, the more uniform and the higher the strength of the gel matrix [220]. Despite $CaCl_2$ is one of the crosslinking agents most frequently used, it leads to rapid and poorly controlled gelation owing to its high solubility in aqueous solution. The use of phosphate buffers enables to slow down and control better the gelation process because phosphate anions compete with the carboxylate groups of alginate in the reaction with Ca^{2+}, retarding the gelation [221]. In addition, less water-soluble calcium salts such as $CaSO_4$ or $CaCO_3$ can reduce the gelation rate as well [222]. Another strategy is to produce the crosslinking at low temperature so as to reduce the reactivity of the crosslinking agent, achieving a slower and more homogeneous gelation [221].

Regarding the viscoelastic properties and the stability of the hydrogel matrix, and the drug release kinetics, it is worth noting that alginate beads exchange the divalent cations from the matrix by non-gelling monovalent ones from the surrounding media, which leads to a rapid dissolution and release of the cargo. Similarly, calcium chelators like phosphate, citrate or lactate destabilize alginate beads [223]. For this reason, several researchers claim that alginate is a “degradable” polymer [224,225]. However, alginate is biostable because humans lack alginate lyase, the enzyme that cleaves the polymer backbone

Table 2
Polymeric nano- and micro-delivery systems developed in ophthalmic administration.

Polymer	Carrier	Drug	Improvement	Cell assay		Ex vivo assay		In vivo assay			Ref
				Type	Cell	Type	Tissue	Type	Administration route	Animal	
Simple											
Alginate	MP	BSA	-Sustained release	-Toxicity	-HCEC	N.D.		N.D.			[38]
	MP	VEGF	-Controlled release	-Toxicity -Bioactivity	-HUVEC	N.D.		N.D.			[39]
	NP	Brimonidine	-Prolonged release -Biological response	-Cytotoxicity	-HEK-293	N.D.		-Pharmacodynamic	Topical	Mouse	[40]
Chitosan	NP	Daptomycin	-Bioactivity	N.D.		N.D.		N.D.			[41]
	NP	Melatonin	-Prolonged release -Residence time	-Cytotoxicity -Permeability -Adhesion	-HCEC	N.D.		N.D.			[42]
	NP	Rosmarinic acid	-Residence time -Permeation	-Cytotoxicity (assay 1) -Permeation (assay 2)	-HCEC (assay 1,2) -RPEC (assay 1,2)	-Tolerance	-Egg	N.D.			[43]
	NP	-	-Residence time *in vivo*	-Toxicity	-Chang	N.D.		-Ocular retention	Topical	Rabbit	[44]
	NP	-	-Uptake	-Toxicity	-IOBANHC	N.D.		-Uptake -Ocular tolerance	Topical	Rabbit	[45]
	MP	BSA or tat-EGFP	-Sustained release -Residence time	-Cytotoxicity	-661W	N.D.		-Ocular toxicity -Ocular tolerance	Sub-retinal	Rat	[46]
	NP	Brimonidine	-Prolonged release -Biological response	-Cytotoxicity	-HEK-293	N.D.		-Pharmacodynamic	Topical	Mouse	[40]
	NP	Norfloxacin	-Residence time	N.D.		-Adhesion (assay 1) -Toxicity (assay 2) -Tolerance (assay 3)	-Goat cornea (assay 1,2) -Egg (assay 3)	N.D.			[47]
	NP	Ketorolac tromethamine	-Prolonged release -Residence time	N.D.		-Permeation	-Porcine cornea	N.D.			[48]
	NP	Dorzolamide	-Prolonged release -Residence time	N.D.		-Adhesion (assay 1) -Permeation (assay 2) -Tolerance (assay 3)	-Goat cornea (assay 1,2) -Egg (assay 3)	-Pre-corneal retention	Topical	Rabbit	[49]
	NP	Resveratrol and quercetin	-Permeation -Biological response	N.D.		-Permeation (assay 1) -Tolerance (assay 2)	-Rabbit cornea (assay 1) -Egg (assay 2)	-Pharmacodynamic	Topical	Rabbit	[50]
	MP	Acyclovir	-Controlled release -Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[51]
	NP	Cyclosporin A	-Prolonged release -Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[52]
	NP	Celecoxib	-Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rat	[53]
Chitosan-PEG	NP	Resveratrol	-Controlled release	N.D.		-Permeation (assay 1) -Tolerance (assay 2)	-Rabbit cornea (assay 1) -Egg (assay 2)	-Pharmacodynamic	Topical	Rabbit	[54]
	NP	Resveratrol and quercetin	-Permeation -Biological response	N.D.		-Permeation (assay 1) -Tolerance (assay 2)	-Rabbit cornea (assay 1) -Egg (assay 2)	-Pharmacodynamic	Topical	Rabbit	[50]
Chitosan-Gal	NP	Timolol	-Sustained release -Residence time -Biological response	N.D.		-Permeation (assay 1) -Tolerance (assay 2)	-Rabbit cornea (assay 1) -Egg (assay 2)	-Preocular retention -Pharmacodynamic	Topical	Rabbit	[55]

(*continued on next page*)

Table 2 (*continued*)

Polymer	Carrier	Drug	Improvement	Cell assay		Ex vivo assay		In vivo assay			Ref
				Type	Cell	Type	Tissue	Type	Administration route	Animal	
TMC (N^+chitosan)	MP	Dexamethasone or tobramycin	-Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[56]
QACD (N^+chitosan)	MP	Rokitamycin	-Controlled release -Mucoadhesion -Biological response *in vitro*	-Toxicity	-HUVEC	N.D.		N.D.			[57]
Thiol-chitosan	NP	Dexamethasone phosphate or metenkephalin	-Residence time	N.D.		N.D.		-Pre-corneal retention	Topical	Rabbit	[58]
Thiol-QACD (SH-N^+chitosan)	NP	Dexamethasone	-Permeation -Bioavailability -Residence time	N.D.		-Permeation -Damage	-Rabbit cornea	-Pharmacokinetic -Ocular irritation	Topical	Rabbit	[59]
Starch acetate	MP	Calcein	-Uptake -Viability	-Uptake	-RPEC	N.D.		N.D.			[60]
CMTKP	NP	Tropicamide	-Residence time	N.D.		-Permeation (assay 1) -Bioadhesion (assay 2) -Irritation (assay 3)	-Goat cornea (assay 1,2) -Egg (assay 3)	N.D.			[61]
Gelatin	NP	Pilocarpine HCl or hydrocortisone	-Sustained release	N.D.		N.D.		N.D.			[62]
	NP	BSA	-Sustained release	-Cytotoxicity	-3T3 FB	N.D.		N.D.			[63]
	NP	-	-Residence time	-Cytotoxicity -Uptake	-HCEC	N.D.		-Ocular distribution -Ocular tolerance	Topical	Rabbit	[64]
	MP	Pilocarpine	-Controlled release -Biological response	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[65]
	NP	Moxifloxacin	-Controlled release -Biological response	N.D.		N.D.		-Pharmacodynamic -Ocular tolerance	Topical	Rabbit	[66]
Ethylcellulose	NP	Acetazolamide	-Controlled release -Permeation -Biological response	N.D.		-Permeation	-Rabbit cornea	-Pharmacodynamic	Topical	Rabbit	[67]
Albumin	NP	Acyclovir	-Permeation	-Cytotoxicity -Permeation	-HCEC	N.D.		N.D.			[68]
	NP	Hydrocortisone	-Permeation -Bioavailability	N.D.		-Permeation	-Porcine cornea	-Pharmacokinetic	Topical	Rabbit	[34]
	NP	Ganciclovir	-Controlled release	N.D.		N.D.		-Ocular disposition -Ocular tolerance	Intravitreal	Rat	[69]
	NP	Carboplatin	-Bioavailability	N.D.		N.D.		-Pharmacokinetic	Posterior sub-tenon	Rat	[70]
	MP	Piroxicam	-Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[71]
	NP	Pilocarpine	-Biological response	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[72]
	MP	Pilocarpine nitrate	-Biological response	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[73]
	MP	Pilocarpine	-Biological response -Controlled release	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[65]
	NP	Curcumin	-Bioavailability	N.D.		N.D.		-Pharmacokinetic -Ocular irritation	Topical	Rabbit	[74]
Albumin-PEG	NP	Apatinib	-Biological response	-Bioactivity	-HUVEC	N.D.		-Distribution -Pharmacodynamic	Sub-conjunctival	Rat	[75]
PCL	MP	Levobunolol HCl	-Sustained release	N.D.		N.D.		N.D.			[76]
	NP	Celecoxib	-Sustained release	-Cytotoxicity	-HEK-293	N.D.		N.D.			[77]
	NP	Loteprednol etabonate	-Controlled release	-Cytotoxicity	-RCEC	N.D.		N.D.			[78]
	NP	Prednisolone	-Controlled release	-Cytotoxicity	-RCEC	-Irritation	-Egg	N.D.			[79]
	NP	Pilocarpine	-Sustained release -Biological response	-Cytotoxicity	-BCEC	N.D.		-Pharmacodynamic	Intracameral	Rabbit	[80]

(*continued on next page*)

Table 2 (*continued*)

Polymer	Carrier	Drug	Improvement	Cell assay		Ex vivo assay		In vivo assay			Ref
				Type	Cell	Type	Tissue	Type	Administration route	Animal	
	NP	Indomethacin	-Uptake -Bioavailability	N.D.		-Uptake	-Rabbit cornea	-Pharmacokinetic	Topical	Rabbit	[81]
	NP	Rhodamine 6G	-Controlled release -Uptake	N.D.		-Uptake -Toxicity	-Rabbit cornea	-Ocular disposition	Topical	Rabbit	[82]
	NP	Celecoxib	-Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rat	[53]
mPEG-PCL	MIC	-	-Biocompatibility	-Cytotoxicity	-HCEC -HLEC -RPEC	N.D.		-Ocular tolerance	Intracameral Intravitreal	Rabbit	[83]
	MIC	Diclofenac	-Sustained release -Permeation -Bioavailability	N.D.		-Permeation	-Rabbit cornea	-Pharmacokinetic -Ocular irritation	Topical	Rabbit	[84]
	MIC	Rapamycin	-Aqueous solubility of the drug -Bioavailability	N.D.		N.D.		-Ocular distribution -Pharmacokinetic -Pharmacodynamic -Biocompatibility	Intravitreal	Rat	[85]
mPEG-PCL-chitosan	MIC	Diclofenac	-Sustained release -Corneal penetration -Bioavailability	-Cytotoxicity	-HCEC -HLEC -L-929	-Permeation	-Rabbit cornea	-Corneal penetration -Pharmacokinetic -Ocular irritation	Topical	Rabbit	[86]
PLA	MP	Rhodamine 6G	-Controlled release -Uptake	-Uptake	-RPEC	N.D.		N.D.			[87]
	NP	Celecoxib	-Sustained release	-Cytotoxicity	-HEK-293	N.D.		N.D.			[77]
	MP	Rhodamine 6G	-Controlled release -Uptake	-Uptake	-RPEC	N.D.		-Uptake -Toxicity	Sub-retinal	Rabbit	[88]
	NP, MP	Triamcinolone acetonide	-Sustained release	-Bioactivity	-ARPE-19	N.D.		-Ocular distribution	Sub-conjunctival	Rat	[89]
	NP	5-Fluorouracil	-Controlled release -Bioavailability -Residence time	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[90]
	MP	TG-0054	-Sustained release	N.D.		N.D.		-Ocular distribution kinetic of MPs	Intravitreal	Rabbit	[91]
	NP	Rhodamine 6G or Nile red	-Controlled release -Ocular disposition of NPs	N.D.		N.D.		-Kinetic of NPs -Ocular toxicity	Intravitreal	Rat	[92]
	MP	5-Fluorouracil	-Controlled release -Ocular disposition of MPs	N.D.		N.D.		-Kinetic of MPs in the vitreous -Ocular toxicity	Intravitreal	Rabbit	[93]
PLA-PEG	NP	Latanoprost acid	-Prolonged release -Bioavailability -Biological response	N.D.		N.D.		-Pharmacokinetic -Pharmacodynamic -Ocular tolerability	Sub-conjunctival	Rabbit	[94]
	MP	Tat-EGFP	-Sustained release -Uptake -Residence time	-Uptake (assay 1) -Cytotoxicity (assay 2)	-661 W (assay1) -HEK-293 (assay 2)	N.D.		-Drug location -Ocular tolerability	Sub-retinal	Rat	[95]
PLA-dextran	NP	Cyclosporine A	-Residence time -Biological response	N.D.		N.D.		-Ocular retention (assay 1) -Pharmacodynamic (assay 2)	Topical	Rabbit (assay 1) Mouse (assay 2)	[96]
Pentablock copolymer: PCL-PLA-PEG-PLA-PCL	NP	IgG-Fab	-Prolonged release	-Cytotoxicity (assay1) -Compatibility (assay2)	-HCEC (assay 1) -RPEC (assay 1) -RAW264.7 (assay2)	N.D.		N.D.			[97]

(*continued on next page*)

Table 2 (*continued*)

Polymer	Carrier	Drug	Improvement	Cell assay		Ex vivo assay		In vivo assay			Ref
				Type	Cell	Type	Tissue	Type	Administration route	Animal	
Pentablock copolymer: PLA-PCL-PEG-PCL-PLA	NP	Peptide/protein	-Sustained release	N.D.		N.D.		N.D.			[98]
Pentablock copolymer: PGA-PCL-PEG-PCL-PGA	NP	Peptide/protein	-Sustained release	N.D.		N.D.		N.D.			[98]
Pentablock copolymer: PEG-PCL-PLA-PCL-PEG	NP	Peptide/protein	-Sustained release	N.D.		N.D.		N.D.			[98]
PLGA	MP	Acyclovir	-Sustained release	N.D.		N.D.		N.D.			[99]
	MP	Vancomycin	-Sustained release	N.D.		N.D.		N.D.			[100]
	MP	Moxifloxacin	-Sustained release	N.D.		N.D.		N.D.			[101]
	MP	Ovalbumin	-Sustained release	N.D.		N.D.		N.D.			[102]
	MP	β-casein or MMP-3	-Sustained release -Protection of the cargo	-Release -Bioactivity	-HTM	N.D.		N.D.			[103]
	NP	Celecoxib	-Sustained release	-Cytotoxicity	-HEK-293	N.D.		N.D.			[77]
	MP	BSA	-Sustained release	-Cytotoxicity	-RSC96 -MC3T3 -L8 -ATDC5	N.D.		N.D.			[104]
	NP	6-Coumarin	-Uptake	-Uptake	-RCjEC	N.D.		N.D.			[36]
	MP	Rhodamine 6G	-Controlled release -Uptake	-Uptake	-RPEC	N.D.		N.D.			[87]
	NP	BSA	-Controlled release -Uptake	-Uptake -Toxicity	-HUVEC	N.D.		N.D.			[105]
	NP	Hydrocortisone butyrate	-Prolonged release	-Uptake -Cytotoxicity	-HCEC	N.D.		N.D.			[106]
	NP	Prodrugs of ganciclovir	-Sustained release	-Cytotoxicity	-HCEC	N.D.		N.D.			[107]
	NP	Ketoconazole	-Controlled release -Permeation	-Permeation	-HCEC	N.D.		N.D.			[108]
	MP	Rhodamine 6G	-Controlled release -Uptake	-Uptake	-RPEC	N.D.		-Uptake -Toxicity	Sub-retinal	Rabbit	[88]
	NP	Cyclosporine A	-Sustained release -Uptake -Bioavailability	-Cytotoxicity -Uptake	-L929	N.D.		-Pharmacokinetic	Topical	Rabbit	[109]
	MP	Celecoxib	-Sustained release	-Cytotoxicity	-ARPE-19 -RF6A	N.D.		-Pharmacodynamic -Safety	Sub-conjunctival	Rat	[110]
	NP	Pranoprofen	-Permeation -Biological response	-Cytotoxicity	-Y-79	-Permeation	-Rabbit cornea	-Pharmacodynamic -Ocular tolerance	Topical	Rabbit	[111]
	NP	Flurbiprofen	-Permeation	N.D.		-Permeation -Corneal damage	-Rabbit cornea	N.D.			[112]
	NP	Loteprednol etabonate	-Penetration	N.D.		-Penetration -Permeation -Ocular tolerance	-Goat cornea	N.D.			[113]
	NP	Flurbiprofen	-Controlled release	N.D.		-Irritation	-Egg	N.D.			[114]
	NP	Pranoprofen	-Sustained release	N.D.		-Tolerance	-Egg	N.D.			[115]
	NP	Acetazolamide	-Permeation -Biological response	N.D.		-Permeation -Corneal toxicity	-Goat cornea	-Ocular irritation -Pharmacodynamic	Topical	Rabbit	[116]
	NP	Dorzolamide	-Sustained release -Permeation -Biological response	N.D.		-Permeation -Ocular distribution -Tolerance	-Goat cornea	-Ocular irritation -Ocular tolerance -Ocular retention -Pharmacodynamic	Topical	Rabbit	[117]

(*continued on next page*)

Table 2 (*continued*)

Polymer	Carrier	Drug	Improvement	Cell assay		Ex vivo assay		In vivo assay			Ref
				Type	Cell	Type	Tissue	Type	Administration route	Animal	
	NP	Sparfloxacin	-Controlled release -Prolonged effect -Permeation -Residence time	N.D.		-Permeation (assay 1) -Irritation (assay 2)	-Goat cornea (assay 1) -Egg (assay 2)	-Ocular retention	Topical	Rabbit	[32]
	NP	Pranoprofen	-Sustained release -Biological response	N.D.		-Permeation (assay 1) -Corneal retention (assay 2) -Tolerance (assay 3)	-Rabbit cornea (assay 1,2) -Egg (assay 3)	-Ocular tolerance -Pharmacodynamic	Topical	Rabbit	[118]
	NP	Carprofen	-Prolonged release -Biological response	N.D.		-Permeation (assay 1) -Retention (assay 2) -Tolerance (assay 3)	-Rabbit cornea (assay 1,2) -Egg (assay 3)	-Ocular irritation -Pharmacodynamic	Topical	Rabbit	[119]
	MP	5-Fluorouracil	-Controlled release	N.D.		N.D.		-Ocular toxicity	Sub-conjunctival	Rabbit	[120]
	NP	Diclofenac Na	-Prolonged release	N.D.		N.D.		-Ocular irritation	Topical	Rabbit	[121]
	NP	Levofloxacin	-Ocular retention	N.D.		N.D.		-Ocular retention	Topical	Rabbit	[122]
	MP	Vancomycin	-Sustained release -Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[123]
	MP	Cyclosporine	-Sustained release *in vitro* and *in vivo*	N.D.		N.D.		-Pharmacokinetic	Intravitreal	Rabbit	[124]
	MP	-	-Residence time *in vivo*	N.D.		N.D.		-Kinetic of MPs in the vitreous -Ocular toxicity	Intravitreal	Rabbit	[125]
	MP	5-Fluorouracil	-Controlled release -Ocular disposition of MPs	N.D.		N.D.		-Kinetic of MPs in the vitreous -Ocular Toxicity	Intravitreal	Rabbit	[93]
	MP	Celecoxib	-Sustained release -Biological response	N.D.		N.D.		-Drug level in ocular tissues -Pharmacodynamic	Sub-conjunctival	Rat	[126]
	MP	Ranibizumab	-Sustained release	N.D.		N.D.		-Ocular safety	Intravitreal	Chick	[127]
	MP	Pilocarpine	-Prolonged effect -Biological response	N.D.		N.D.		-Mucoadhesion -Pharmacodynamic -Ocular safety	Topical	Rabbit	[128]
	MP	Albumin (model protein) or doxycycline	-Sustained release -Biological response	N.D.		N.D.		-Ocular distribution -Pharmacodynamic -Ocular safety	Sub-conjunctival	Mouse	[129]
	MP	Brimonidine	-Controlled release -Efficacy	N.D.		N.D.		-Preocular retention -Safety -Pharmacodynamic	Topical	Rabbit	[130]
	MP	Vancomycin	-Controlled release -Biological response	N.D.		N.D.		-Pharmacokinetic -Pharmacodynamic -Ocular toxicity	Intracameral	Rabbit	[131]
	MP	Dexamethasone	-Sustained release -Biological response	N.D.		N.D.		-Pharmacodynamic	Intravitreal	Rabbit	[132]
	NP	Dexamethasone	-Sustained release -Bioavailability -Biological response	N.D.		N.D.		-Retention of carriers -Pharmacokinetic -Pharmacodyinamic -Ocular safety	Sub-conjunctival	Rat	[133]
	NP	Brinzolamide	-Sustained release -Biological response	N.D.		N.D.		-Pharmacodynamic -Compatibility	Sub-conjunctival	Rabbit	[134]
PLGA-TPGS	NP	Dexamethasone	-Sustained release -Bioavailability	N.D.		N.D.		-Pharmacokinetic	Intravitreal	Rabbit	[135]

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Table 2 (*continued*)

Polymer	Carrier	Drug	Improvement	Cell assay		Ex vivo assay		In vivo assay			Ref
				Type	Cell	Type	Tissue	Type	Administration route	Animal	
POE	NP	Epinephrine	-Sustained release	-Cytotoxicity (assay 1) -Uptake (assay 2)	-HEK-293 (assay 1,2) -Müller (assay 2)	N.D.		N.D.			[136]
	NP	Celecoxib	-Sustained release	-Cytotoxicity (assay 1) -Uptake (assay 2)	-HEK-293 (assay 1,2) -Müller (assay 2)	N.D.		N.D.			[137]
	NP	Nile red	-Ocular distribution	N.D.		N.D.		-Ocular localization (assay 1) -Ocular safety (assay 2)	Intravitreal	Mouse (assay 1) Rabbit (assay 2)	[138]
Eudragit® RL	NP	Sulfacetamide	-Controlled release	N.D.		N.D.		N.D.			[139]
Eudragit® RL, RS and blends	MP	Acetazolamide	-Sustained release	N.D.		N.D.		N.D.			[140]
Eudragit® RS or RL	NP	Cloricromene	-Sustained release -Protection of the cargo	N.D.		N.D.		N.D.			[141]
Eudragit® RS + RL	MP	Gentamicin	-Controlled release -Biological response *in vitro*	N.D.		N.D.		N.D.			[142]
Eudragit® RL, RS, NE and blends	MP	Acyclovir	-Sustained release	-Bioactivity	-VERO cells infected with HSV	N.D.		N.D.			[143]
Eudragit® RS and RL	NP	Gatifloxacin	-Prolonged release	-Cytotoxicity	-HCEC	N.D.		N.D.			[144]
Eudragit® RS	NP	Prednisolone	-Controlled release	-Cytotoxicity	-RCEC	-Irritation	-Egg	N.D.			[79]
Eudragit® RL	NP	Moxifloxacin	-Sustained release -Permeation	N.D.		-Permeation -Irritation	-Goat cornea	N.D.			[145]
Eudragit® RL	NP	Ketotifen fumarate	-Sustained release	N.D.		-Permeation	-Bovine cornea	N.D.			[146]
Eudragit® RL	NP	Aceclofenac	-Sustained release -Permeation -Biological response	N.D.		-Permeation	-Goat cornea	-Pharmacodynamic	Topical	Rabbit	[147]
Eudragit® RL	NP	Ketoroloac tromethamine	-Controlled release -Mucoadhesion -Permeation -Bioavailability	N.D.		-Permeation	-Bovine cornea	-Pharmacokinetic -Ocular irritation	Topical	Rabbit	[148]
Eudragit® RL	NP	Acetazolamide	-Controlled release -Permeation -Biological response	N.D.		-Permeation	-Rabbit cornea	-Pharmacodynamic	Topical	Rabbit	[67]
Eudragit® RS	NP	Acetazolamide	-Controlled release -Permeation -Biological response	N.D.		-Permeation	-Goat cornea	-Pharmacodynamic -Ocular tolerance	Topical	Rabbit	[25]
Eudragit® RS or RL	NP	-	-Ocular tolerability	N.D.		N.D.		-Toxicity	Topical	Rabbit	[23]
Eudragit® RS	NP	Terbinafine.HCl	-Controlled release -Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[149]
Eudragit® RL	NP	Pilocarpine	-Biological response	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[150]
Eudragit® RL	NP	Acetazolamide	-Biological response	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[151]
Eudragit® RS or RL	NP	Flurbiprofen	-Prolonged release -Biological response	N.D.		N.D.		-Ocular irritation -Pharmacokinetic -Pharmacodynamic -Toxicity	Topical	Rabbit	[152]
Eudragit® RS and RL	NP	Brimonidine Tartrate	-Controlled release -Biological response	N.D.		N.D.		-Ocular irritation -Pharmacodynamic	Topical	Rabbit	[153]
Eudragit® RS	NP	MPA	-Controlled release	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[154]

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Table 2 (*continued*)

Polymer	Carrier	Drug	Improvement	Cell assay		Ex vivo assay		In vivo assay			Ref
				Type	Cell	Type	Tissue	Type	Administration route	Animal	
Eudragit® RS	NP	Piroxicam	-Controlled release -Biological response	N.D.		N.D.		-Pharmacodynamic -Ocular tolerance	Topical	Rabbit	[24]
Eudragit® RS	NP	Cyclosporine A	-Prolonged release -Residence time	N.D.		N.D.		-Pharmacokinetic	Topical	Sheep	[155]
Eudragit® RS	NP	Ibuprofen Na	-Biological response	N.D.		N.D.		-Pharmacocinetic -Pharmacodynamic	Topical	Rabbit	[156]
Eudragit® RS	NP	Ibuprofen Na	-Prolonged release -Biological response	N.D.		N.D.		-Ocular irritation -Pharmacokinetic -Pharmacodynamic -Toxicity	Topical	Rabbit	[157]
Eudragit® RL	NP	Cloricromene	-Sustained release -Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[27]
Eudragit® RL	NP	Amphotericin B	-Prolonged release	N.D.		N.D.		-Ocular irritation	Topical	Rabbit	[158]
Eudragit® S	NP	Diclofenac	-Sustained release -Biological response	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[159]
PACA	NP	-	-Uptake	N.D.		-Uptake	-Rabbit cornea -Rabbit conjunctiva	N.D.			[160]
	NP	Acyclovir	-Bioavailability	N.D.		N.D.		-Ocular irritation -Pharmacokinetic	Topical	Rabbit	[161]
	NP	-	-Pre-ocular retention	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[162]
	NP	-	-Pre-ocular retention	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[35]
	NP	-	-Pre-ocular retention	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[163]
	NP	Ganciclovir	-Controlled release -Bioavailability	N.D.		N.D.		-Pharmacokinetic -Ocular toxicity	Intravitreal	Rabbit	[164]
	NP	Amikacin.H_2SO_4	-Ocular disposition	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[165]
	NP	Pilocarpine	-Biological response	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[166]
	NP	Betaxolol.HCl	-Controlled release -Pharmacodynamic	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[167]
PAA	NP	Brimonidine	-Controlled release -Ashesion	-Toxicity -Adhesion -Permeation	-HCEC	N.D.		N.D.			[168]
	MP	-	-Mucoadhesion -Residence time *in vivo*	N.D.		N.D.		-Precorneal clearance of microspheres	Topical	Rabbit	[169]
p(NiPAAm-MAA-VP)	NP	Ciprofloxacin	-Biological response	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[170]
PEA	MP	Dexamethasone	-Sustained release	-Cytotoxicity	-RAW264.7 -RPEC	N.D.		-Ocular location of microparticles	Sub-tenon and intravitreal	Rat	[171]
PCP	MP	Sulfacetamide Na	-Controlled release -Mucoadhesion -Biological response *in vitro* and *in vivo*	N.D.		N.D.		-Pharmacodynamic	Topical	Rabbit	[172]
PS	NP, MP	-	-Retention	N.D.		N.D.		-Retention of carriers -Ocular distribution	Sub-conjunctival	Rat	[173]
PS-PEG	NP	-	-Distribution of NPs	N.D.		-Distribution of NPs	-Bovine vitreous	N.D.			[174]
Hybrid											
Alginate and chitosan	NP	Gatifloxacin	-Controlled release	N.D.		N.D.		N.D.			[175]
Alginate and chitosan	NP	Daptomycin	-Permeation	-Permeation	-HCEC -ARPE-19	N.D.		N.D.			[176]
Alginate and chitosan	MP	Indomethacin	-Controlled release	-Cytotoxicity	-3T3 FB	N.D.		N.D.			[177]

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Table 2 (*continued*)

Polymer	Carrier	Drug	Improvement	Cell assay		Ex vivo assay		In vivo assay			Ref
				Type	Cell	Type	Tissue	Type	Administration route	Animal	
Alginate and chitosan	NP	5-fluorouracil	-Sustained release -Permeation -Residence time -Bioavailability	N.D.		-Permeation	-Rabbit cornea	-Pharmacokinetic -Ocular irritation	Topical	Rabbit	[178]
Alginate and chitosan	NP	Betamethasone sodium phosphate	-Sustained release -Permeation -Bioavailability	N.D.		-Permeation	-Rabbit sclera	-Pharmacokinetic	Topical	Rabbit	[179]
Alginate and chitosan	MP	Azelastine	-Controlled release -Mucoadhesion -Biological response	N.D.		N.D.		-Pharmacodynamic	Topical	Rat	[180]
Alginate and thiolated-chitosan	NP	FITC	-Mucoadhesion -Ocular delivery	-Cytotoxicity -Uptake	-HCEC	N.D.		-Ocular delivery	Topical	Rat	[181]
Chitosan and albumin	MP	Atropine sulfate	-Uptake -Biological response	-Cytotoxicity -Uptake -Localization	-HCEC	N.D.		-Pharmacodynamic	Topical	Rabbit	[182]
Chitosan and albumin	MP	Tetracaine HCl	-Controlled release -Uptake -Prolonged effect	-Cytotoxicity -Uptake -Localization	-HCEC	N.D.		-Pharmacodynamic	Topical	Rabbit	[183]
Chitosan and gelatin	NP	-	-Ocular distribution	N.D.		N.D.		-Ocular distribution	Intravitreal	Rat	[184]
Chitosan and gelatin	NP	Cefuroxime	-Sustained release	N.D.		N.D.		-Ocular distribution -Drug release	Intravitreal	Rat	[185]
Chitosan and chondroitin sulfate	NP	Bromfenac Na	-Sustained release -Permeation -Residence time -Cellular uptake	N.D.		-Permeation (assay 1) -Retention (assay 2) -Uptake (assay 3) -Tolerance (assay 4)	-Goat cornea (assay 1,2,3) -Egg (assay 4)	N.D.			[186]
Chitosan and dextran sulfate	NP	Lutein	-Residence time -Drug stability	N.D.		N.D.		N.D.			[187]
Chitosan and dextran sulfate	NP	-	-Mucoadhesion -Uptake	-Uptake	-PCEC	-Adhesion -Penetration	-Porcine cornea	N.D.			[188]
Gelatin and dextran sulfate	NP	Plasmid DNA	-Protection of the cargo -Uptake -Transfection efficiency	-Cytotoxicity -Uptake -Transfection efficiency	-HCEC	N.D.		N.D.			[37]
Gelatin and dextran sulfate	NP	pMUC5AC	-Transfection efficiency	-Cytotoxicity -Transfection efficiency	-IOBA-NHC -HCEC	N.D.		-Transfection efficiency	Topical	Rabbit	[189]
Cationized gelatin,SPM, chondroitin sulfate	NP	siRNA	-Uptake	-Cytotoxicity -Transfection efficiency -Uptake	-HCEC	N.D.		-Ocular distribution	Topical	Rabbit	[190]
Gelatin and chondroitin sulfate	NP	Plasmid DNA	-Cargo protection -Uptake	-Cytotoxicity -Uptake -Transfection efficiency	-HCEC	N.D.		N.D.			[37]
Gelatin and chondroitin sulfate	NP	pMUC5AC	-Transfection efficiency	-Cytotoxicity -Transfection efficiency	-IOBA-NHC -HCEC	N.D.		-Transfection efficiency	Topical	Rabbit	[189]
Chitosan and HPMC	NP	Sparfloxacin	-Biological response	-Cytotoxicity	-VERO	-Irritation	-Egg	-Pharmacodyinamic	Topical	Rabbit	[191]
Chitosan and HA	NP	Dexamethasone sodium phosphate	-Controlled release	N.D.		N.D.		N.D.			[192]

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Table 2 (*continued*)

Polymer	Carrier	Drug	Improvement	Cell assay		Ex vivo assay		In vivo assay			Ref
				Type	Cell	Type	Tissue	Type	Administration route	Animal	
Chitosan and HA	NP	pEGFP or pβ-gal	-Uptake	-Uptake -Transfection -Toxicity	-HCEC -IOBA-NHC	N.D.		N.D.			[193]
Chitosan and HA	NP	Dexamethasone sodium phosphate	-Bioavailability	N.D.		-Permeation	-Rabbit cornea	-Precorneal drug kinetics -Pharmacokinetic -Ocular irritation	Topical	Rabbit	[194]
Chitosan and HA	NP	5-Fluorouracil	-Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[195]
Albumin and HA	NP	Cx43 MP	-Sustained release -Uptake -Penetration	-Cytotoxicity -Active Targeting	-ARPE-19	-Penetration	-Bovine retina	N.D.			[196]
Albumin and HA	NP	-	-Penetration	N.D.		-Penetration -Damage	-Bovine eye cups	N.D.			[197]
PCL and chitosan	NP	Indomethacin	-Ocular distribution -Bioavailability	N.D.		N.D.		-Ocular distribution -Pharmacokinetic -Ocular toxicity	Topical	Rabbit	[198]
PCL and chitosan	NP	Coumarin 6	-Penetration -Bioavailability	-Cytotoxicity	-SIRC	N.D.		-Ocular penetration	Topical	Mouse	[199]
PCL and gelatin	NP	Coumarin 6	-Penetration -Bioavailability	-Cytotoxicity	-SIRC	N.D.		-Ocular penetration	Topical	Mouse	[199]
PCL and PF68	NP	Coumarin 6	-Penetration -Bioavailability	-Cytotoxicity	-SIRC	N.D.		-Ocular penetration	Topical	Mouse	[199]
PCL and Eudragit® RS	NP	Vancomycin	-Controlled release -Biological response -Residence time	N.D.		N.D.		-Biological effect -Ocular toxicity	Topical	Rabbit	[200]
PLA and chitosan	NP	5-Fluorouracil	-Controlled release -Residence time -Permeation -Bioavailability	N.D.		-Permeation	-Goat cornea -Rabbit cornea	-Pharmacokinetic -Irritation	Topical	Rabbit	[201]
PLGA and chitosan	NP	Pilocarpine	-Mucoadhesion -Sustained release	N.D.		N.D.		N.D.			[202]
PLGA and chitosan	NP	Fluocinolone acetonide	-Sustained release -Residence time -Bioavailability	N.D.		N.D.		-Ocular irritation -Pharmacokinetic	Topical	Rabbit	[203]
PLGA and chitosan	NiMDS	Ranibizumab	-Sustained release	-Cytotoxicity -Bioactivity	-ARPE-19 -HUVEC	N.D.		N.D.			[204]
PLGA and chitosan	NP	Bevacizumab	-Sustained release -Mucoadhesion -Permeation	N.D.		-Permeation (assay 1) -Tolerance (assay 2)	-Goat sclera (assay 1) -Egg (assay 2)	N.D.			[205]
PLGA and chitosan	NP	Fluorescent rhodamine	-Residence time -Uptake	N.D.		-Retention -Uptake -Irritation	-Goat cornea	-Retention -Uptake -Ocular tolerance	Topical	Rabbit	[206]
PLGA and alginate	MP	BSA	-Sustained release	-Cytotoxicity	-RSC96 -MC3T3 -L8 -ATDC5	N.D.		N.D.			[104]
PLGA and albumin	NP	Bevacizumab	-Sustained release -Bioavailability	N.D.		N.D.		-Ocular distribution -Pharmacokinetic -Ocular toxicity	Intravitreal	Rabbit	[207]
PLGA and Eudragit® RL	NP	Cyclosporine A	-Sustained release -Uptake -Bioavailability	-Cytotoxicity -Uptake	-L929	N.D.		-Pharmacokinetic	Topical	Rabbit	[109]

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Table 2 (*continued*)

Polymer	Carrier	Drug	Improvement	Cell assay		Ex vivo assay		In vivo assay			Ref
				Type	Cell	Type	Tissue	Type	Administration route	Animal	
PLGA and Carbopol®	NP	Cyclosporine A	-Sustained release -Uptake -Bioavailability	-Cytotoxicity -Uptake	-L929	N.D.		-Pharmacokinetic	Topical	Rabbit	[109]
PCL and HA	NP	Cyclosporine A	-Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[208]
Eudragit® RL, RS and HA	NP	Gatifloxacin and prednisolone	-Controlled release -Bioavailability	N.D.		N.D.		-Pharmacokinetic	Topical	Rabbit	[209]
PLA and gelatin	MP	POPOP	-Uptake	-Uptake	-RPEC	N.D.		N.D.			[210]
PLGA and PEG	MP	Rhodamine B or nile red	-Mucoadhesion -Residence time *in vivo*	N.D.		N.D.		-Retention of carriers	Topical	Rabbit	[211]
CMC and PVA	MP	Adrenalin	-Sustained release -Biological response	N.D.		N.D.		-Pharmacodynamic	Topical	Human	[212]
PLGA and PLA	NIMDS	Bevacizumab	-Sustained release -Prolonged delivery	N.D.		N.D.		-Ocular drug distribution	Intravitreal	Rat	[213]
PLGA and PLA	NP	Erythropoietin	-Ocular tolerability	N.D.		N.D.		-Ocular safety	Intravitreal	Rabbit	[214]

PEG = poly(ethylene glycol), Chitosan-Gal = galactosylated chitosan, TMC = N-trimethyl chitosan, QACD = quaternary ammonium chitosan derivatives, CMTKP = carboxymethyl tamarind kernel polysaccharide, PCL = poly(epsilon-caprolactone), mPEG = methoxypoly(ethylene glycol), PLA = poly (D, L-lactic acid), PLGA = poly(lactic-co-glycolic) acid, PLGA-TPGS = poly(lactide-coglycolide)-D-α-tocopheryl polyethylene glycol 1000 succinate, POE = poly(ortho ester), PACA = poly(alkyl cyanoacrylate), PAA = polyacrylic acid, P(NiPAAm-MAA-VP) = Poly(N-isopropylacrylamide-methacrylic acid-vinylpyrrolidone), PEA = poly(ester amide)s, PCP = Polycarbophil (polymer of polyacrylic acid cross-linked with divinyl glycol), PS = Polystyrene, SPM = spermine, HPMC = hydroxypropylmethylcellulose, HA = hyaluronic acid, PF68 = Pluronic® F68, CMC = carboxymethylcellulose, PVA = poly(vinyl alcohol).

NP = Nanoparticle, MP = Microparticle, MIC = micelles, NIMDS = nanoparticle-in-microparticle delivery system.

BSA = bovine serum albumin, VEGF = vascular endothelial growth factor, Tat-EGFP = enhanced green fluorescent protein fused to the transactivator of transcription peptide, MMP-3 = Matrix metalloproteinase-3, MPA = methylprednisolone acetate, FITC = fluorescein isothiocyanate, pMUC5AC = plasmid codified for a modified MUC5AC protein, pEGFP = Plasmid DNA encoding green fluorescent protein, pβ-gal = Plasmid DNA encoding β-galactosidase, Cx43 MP = 43 kDa connexin isoform mimetic peptide, POPOP = 1,4-bis[2-(5-phenyloxazolyl)]-benzene.

N.D. = not determined, HCEC = human corneal epithelial cells, HUVEC = human umbilical vein endothelial cells, HEK-293 = Human embryonic kidney cells 293, RPEC = retinal pigment epithelial cells, Chang = comercial Chang conjunctival cell line, IOBA-NHC = immortalized human conjunctival epithelial cell line, 661 W = mouse photoreceptor-derived 661 W cells, FB = fibroblasts, RCEC = Rabbit corneal epithelial cells, BCEC = bovine corneal endotelial cells, HLEC = human lens epithelial cells, L929 = mouse fibroblast cells, ARPE-19 = human retinal pigment epithelial cells, RAW264.7 = mouse macrophages, HTM = human trabecular meshwork cells, RSC96 = Schwann cell derived cell line, MC3T3 = fibroblast-like cell line, L8 = skeletal muscle cell line, ATDCS = chondrocyte cell line, RCjEC = Rabbit conjunctival epithelial cells, RF6A = monkey choroid–retinal endothelium, Y-79 = Human retinoblastoma cell line, Muller cells = retinal glial cells, PCEC = porcine corneal epithelial cells, SIRC = corneal epithelial cell line.

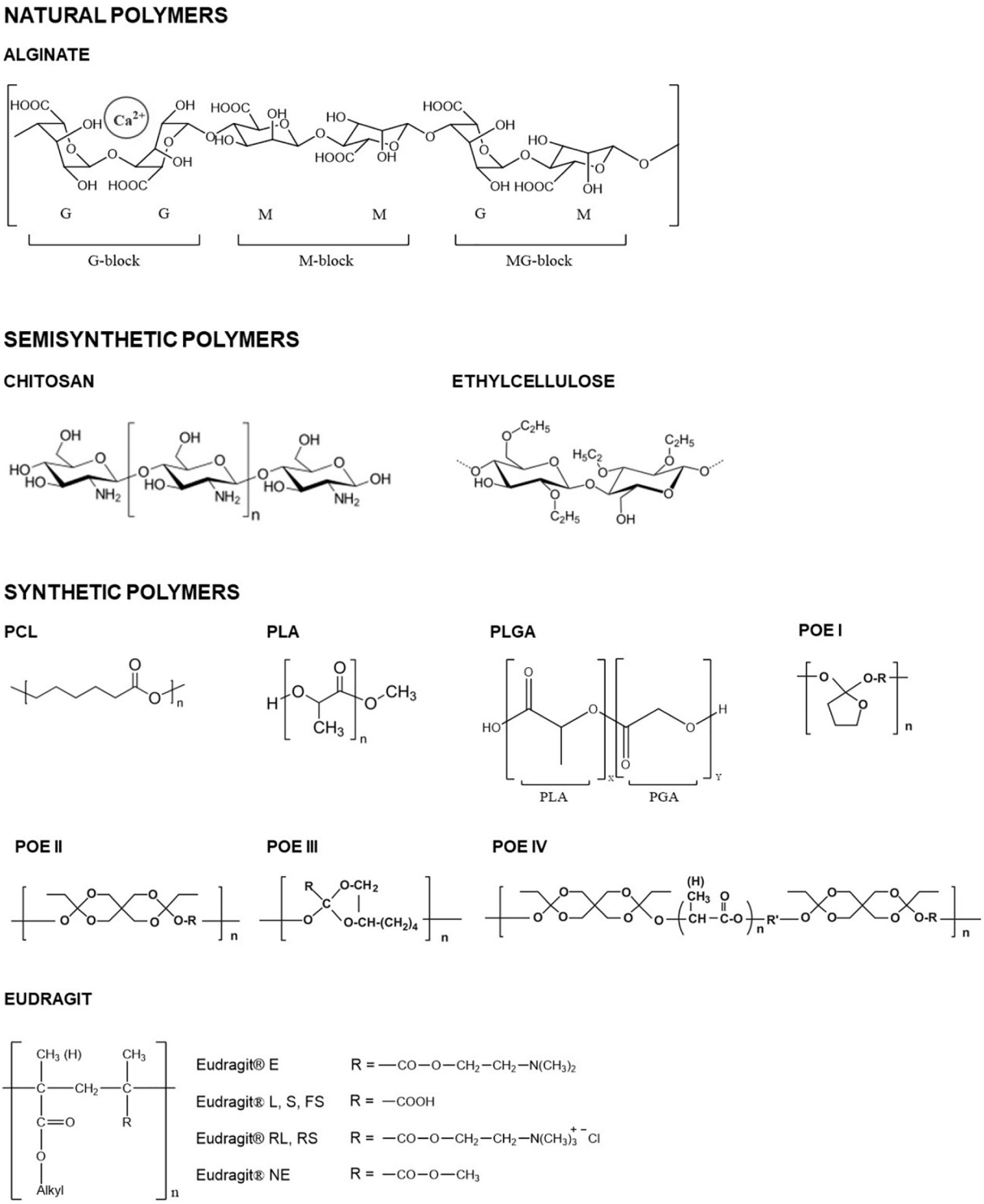

Fig. 2. Chemical structure of polymers used in the development of ocular drug delivery systems.

[226]. Instead, the presence of calcium chelators and non-gelling cations that compete with multivalent ones dissolve the gel. Phenomena that trigger the dissolution of the beads are much slower in the cul-de-sac compared to other eye sites due to the low volume of the lachrymal fluid in the human eye (7.5-10 μL) [7]. It is worth noting that alginate has a large amount of hydroxyl groups to form H bonds with carboxyl groups of mucin glycoproteins, the main component of the inner layer of the ocular mucosa. This interaction can prolong the residence time of alginate carriers. Furthermore, due to its hydrophilic nature, alginate swells in aqueous media allowing maximal exposure of potential anchor sites and adopting maximal distance between the chains leading to increased chain flexibility and effective interpenetration with the mucus layer. The typical high molecular weight of alginate further contributes to mucoadhesion [227]. Consequently, several alginate-based carriers were developed for ophthalmic drug adminisration [38–40,53,228]. For example, brimonidine-loaded alginate nanoparticles led to a 2.8-fold increase of the area under intraocular pressure (IOP) curve with respect to commercial eye drops (Alphagan P®) in BXD29 mice with spontaneously elevated IOP. The time required to reach maximum decrease in IOP and the duration of the drug effect

were 2.2 and 5.8 h for commercial eye drops and 7.0 and 21.6 h for alginate nanoparticles, respectively, revealing the beneficial role of this mucoadhesive polymer [40].

The low stability and fast disintegration of alginate matrices in the biological environment still represents a disadvantage owing to the relatively fast release of the cargo. Several strategies were applied to slow down the drug release kinetics. The simplest is the incorporation of alginate particles to bioadhesive vehicles. For example, Liu *et al.* developed a collagen hydrogel containing bovine serum albumin (BSA)-loaded alginate microspheres for ocular drug delivery [38]. These composite delivery systems released the drug during an 11-day period in neutral buffer without the typical burst effect of calcium alginate beads. These results could be explained by the two barriers that BSA has to permeate for the release: first the microsphere and second the collagen matrix [38]. This approach is more thoroughly discussed below. The production of a hybrid polymer carrier is another strategy used to improve the stability of alginate platforms. The most common combination is with the semi-synthetic polycationic polymer chitosan (see the corresponding section for a more detailed description of this polymer). These polyelectrolytic complexes are stable in the presence of calcium and show reduced porosity, reducing the diffusion rate of the encapsulated drug. On this front, it is possible to coat alginate particles with chitosan [176,179] or to form a hybrid alginate/chitosan matrix [175,180]. This hybrid matrix can be coated with an additional polymeric barrier to control or delay even more the release [178,229]. Achievements reached with hybrid polymer carriers will be discussed later.

Another key factor of alginate structure is the presence of functional groups; two secondary −OH and one −COOH per repeating unit that can be modified to improve the physical properties of the polymer [219]. However, this is an unexplored field in ocular administration.

One of the most remarkable advantages of alginate is its biocompatibility. For instance, it is listed as “Generally Recognized as Safe” (GRAS) by the US-Food and Drug Administration (US-FDA) [230]. Thus, it is widely used in food and topical and oral pharmaceutical products [231]. However, the eye is an especially sensitive organ and thus, the toxicity of alginate particles must be assessed. Several studies demonstrated the good cytocompatibility of alginate in different cell lines *in vitro*. Additionally, an aqueous solution of alginate 1% w/v was non-irritant by the Draize test that is a technique based on the observation of residual injury signs after the instillation of the potential irritant samples in the conjunctival sac of rabbits and observation for 96 h [232]. Remarkably, no ocular damage or abnormal clinical signs were visible in to cornea, iris or conjunctivae for 7 days [233]. Likewise, this alginate concentration did not show irritation by the Hen's Egg Test Chorioallantoic Membrane (HET-CAM) or cytotoxicity by using a novel method for real-time monitoring complemented with the cell proliferation assay WST-1 [234]. The former is an *ex vivo* method alternative to the Draize test, based on vascular changes in the HET-CAM that is used as an analog of the ocular conjunctiva, in response to an irritant substance [235]. The latter relies on a reduction of normal cell index due to changes in the number or the morphology of the cells caused by the addition of potentially toxic agents [234]. Importantly, since alginate is extracted from natural sources, a number of impurities, including proteins, could be present eventually causing immunogenic reactions. However, this should not be a concern when using commercial alginate because it is highly purified [236]. Beyond the biocompatibility of the polymer, its administration as particles should be evaluated. Ibrahim *et al.* showed that alginate NPs of approximately 100 nm alone and once incorporated in eye drops, *in situ* gelling systems and preformed gels were nontoxic as determined by the methyl thiazol tetrazolium (MTT) assay using a polymer concentration of 0.6 % w/v in each vehicle and the human embryonic kidney cell line HEK293 [40]. The toxicity of the crosslinking agent has to be evaluated as well. For example, Ca^{2+}-crosslinked particles were less cytotoxic than those prepared with Zn^{2+} and Ba^{2+} [39]. In addition, organic solvent-free production methods can be used to prepare alginate NPs with improved biocompatibility. Finally, as mentioned above, alginate ODDS must be sterilized. Autoclaving [237–239], ethylene oxide [237,240] and UV-irradiation [239,241] of both alginate powder and hydrogel lead to scission of the chain polymer with a reduction of its molecular weight and viscosity. These changes have a negative impact on the mechanical properties of alginate hydrogel beads formed from these solutions. Thus, an appropriate production method that ensures the properties of the product has to be developed.

2.1.2. Albumin

Albumin is a polyanionic natural protein isolated from a broad range of sources such as egg white (ovalbumin, OVA), bovine (BSA) and human plasma (human serum albumin, HSA). Its biological function is related to the maintenance of the osmotic pressure and the transport of nutrients to cells. Furthermore, a plethora of drugs bind albumin and their transport and bioavailability are altered. Albumin is constituted by a single polypeptide chain of 585 amino acids, including a low content of tryptophan and methionine and a large amount of cysteine, aspartic acid, glutamic acid, lysine and arginine. Its isoelectric point is approximately 4.7. Accordingly, at physiological pH, albumin has a net electrostatic charge of about -17 mV [231,242]. Albumin is biodegradable and has functional groups that can be used to bind different ligands and complex drugs (e.g., paclitaxel in Abraxane®) [243]. All these characteristics empower albumin as a carrier candidate for ocular administration. In fact, it has been demonstrated that following the instillation of pilocarpine-loaded OVA MPs with a size > 30 μm to rabbits, a greater biological response over a longer time was obtained with respect to both the drug aqueous solution and a viscous aqueous solution [65]. Equally, BSA microspheres with size > 10 μm containing piroxicam caused 1.8 fold-increase in bioavailability after its topical administration in the eye to rabbits when compared to the standard commercial eyedrops [71]. Outstandingly, Rathod *et al.* showed that a higher biological response was obtained after topical administration of pilocarpine nitrate-loaded OVA MPs with a drug concentration of 1% w/v than with pilocarpine nitrate solutions in the 1-4% w/v concentration range [73]. It has also been established that the retention of albumin NPs was higher in inflamed eyes than in healthy tissue due to the presence of the enhanced permeation and retention (EPR) effect [34]. Beyond the topical application, other ocular administration routes have been studied to evaluate the performance of albumin particles. For example, HSA-poly(ethylene glycol) (PEG) was used to nanoencapsulate apatinib, a water-insoluble angiogenesis inhibitor [75]. After its subconjunctival administration, a significant decrease in neovascularization compared to the one observed with an injection of a drug solution was reached [75]. Even, a large number of NPs was detected in the corneal stroma 24 h after injection [75]. Merodio *et al.* injected 300 nm biotin-labeled BSA NPs to rats by intravitreal injection [69]. Two weeks post-injection, a significant amount of them remained in the vitreous cavity, especially in a thin layer overlying the retina, although they were also located in the iris and ciliary body. To enhance the permeation of nanoparticles across the retina after intravitreal injection, Huang *et al.* proposed the application of ultrasound [197]. As seen in Fig. 3A, a large amount of FITC-loaded hyaluronic acid coated HSA NPs remained at the injection site after 5 min without ultrasound application. On the contrary, NPs diffused away from the injection site to the surrounding vitreous when triple trasnsscleral ultrasound was applied. Additionally, a significant increase in the distribution area and decrease in the fluorescence intensity at the original injection site were observed after ultrasound application (Fig. 3B) [197].

Carboplatin-loaded BSA NPs were injected via a posterior subtenon route [70]. The vitreal concentration of drug was much higher (mean concentration = 11.66 μg/mL) with NPs compared to the commercially available carboplatin formulation (mean concentration = 1.17 μg/mL) in the first week of the study. Conversely, this relationship was reversed on day 14 with a mean concentration of 10.49 and 1.67 μg/mL for free

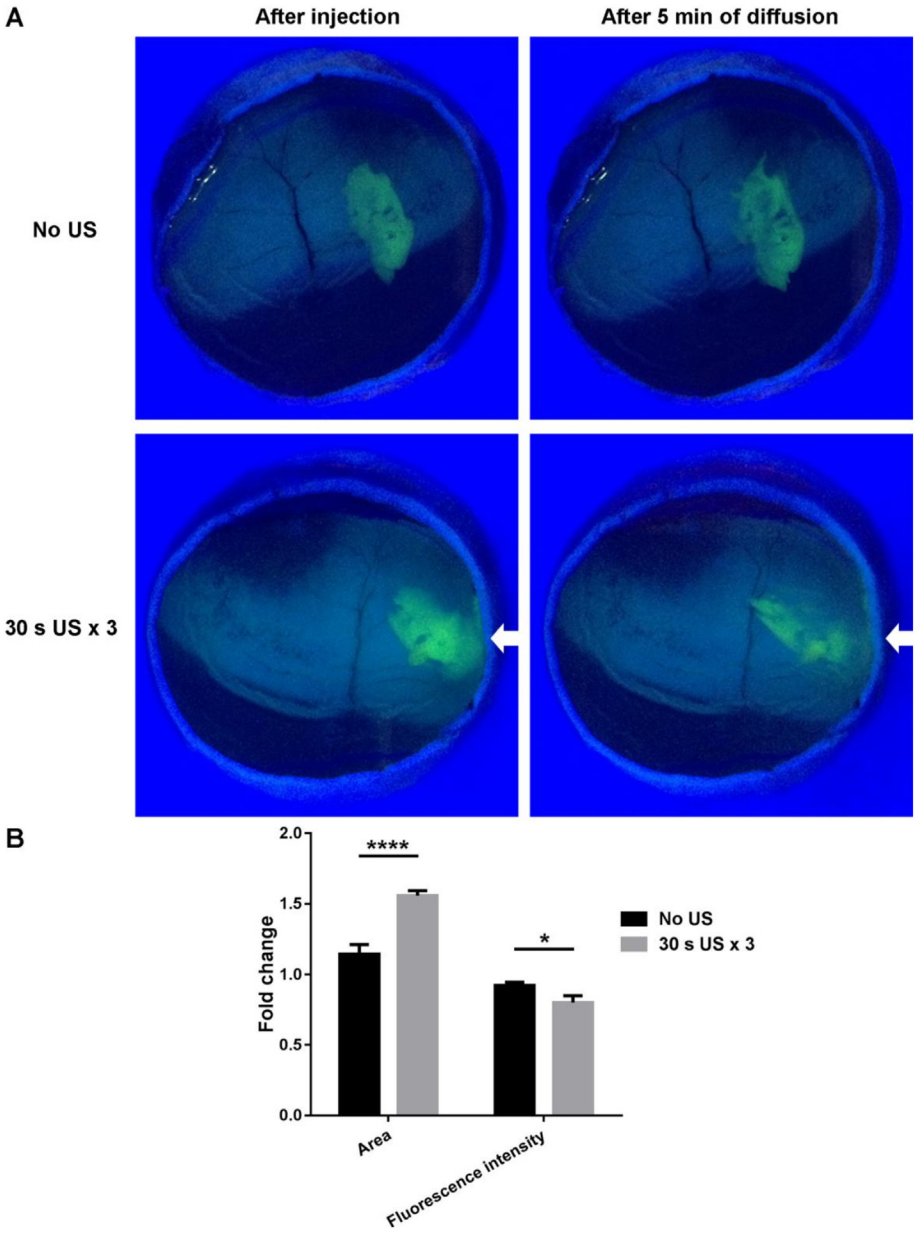

Fig. 3. Nanoparticle diffusion in intact bovine vitreous *ex vivo* without or with ultrasound application. (A) Nanoparticle distribution in the vitreous immediately after intravitreal injection (left) and after 5 min of diffusion (right), without (top) and with triple transscleral ultrasound application (bottom). White arrows mark the site of ultrasound application. (B) Quantification of the fold change in fluorescent area and intensity using ImageJ software (data points represent mean values ± S.D., n = 3). Reproduced with permission of Elsevier Science from Huang *et al.* [197].

carboplatin and carboplatin-loaded NPs, respectively [70]. Authors explained that the small size of the ODDS causes a stronger osmotic gradient for transscleral migration initially into the vitreous, while this molecule is probably transported out of the vitreous at an earlier stage than the conventional molecule [70].

Regarding the safety profile of albumin, it is important to emphasize that this protein is biodegradable and biocompatible. In fact, it is naturally produced in the body. Albumin is used primarily in parenteral formulations [231]. No cytotoxic effect on human corneal epithelial (HCE) cell multilayer, an *in vitro* model of human cornea, were evidenced with aciclovir-loaded BSA NPs [68]. Furthermore, a thermoresponsive ophthalmic *in situ*-forming gel containing curcumin-loaded BSA NPs was not irritant as determined by the Draize test [74]. Also, ganciclovir-loaded BSA NPs did not generate changes in the cyto-architecture of the retina and the surrounding tissues after its injection in rats [69]. Interestingly, these NPs did not alter the expression and distribution of arrestin and rhodopsin autoantigens suggesting that they do not generate an autoimmune response [69].

2.2. Semisynthetic polymers

2.2.1. Chitosan

Chitosan is a semisynthetic polycationic polysaccharide obtained by chemical deacetylation of chitin, the second most abundant polymer in nature after cellulose that is mainly isolated from crustaceans [244]. Structurally, chitosan is a linear unbranched polymer consisting of β-(1→4) linked D-glucosamine with randomly located *N*-acetylglucosamine groups depending on the level of deacetylation of the polymer, ranging from 66% to 95% (Fig. 2). Its composition, molecular weight and purity varies depending on the source, the extraction and processing method and the manufacturer [245]. Thus, there is a wide range of commercially available chitosan products of different degree of

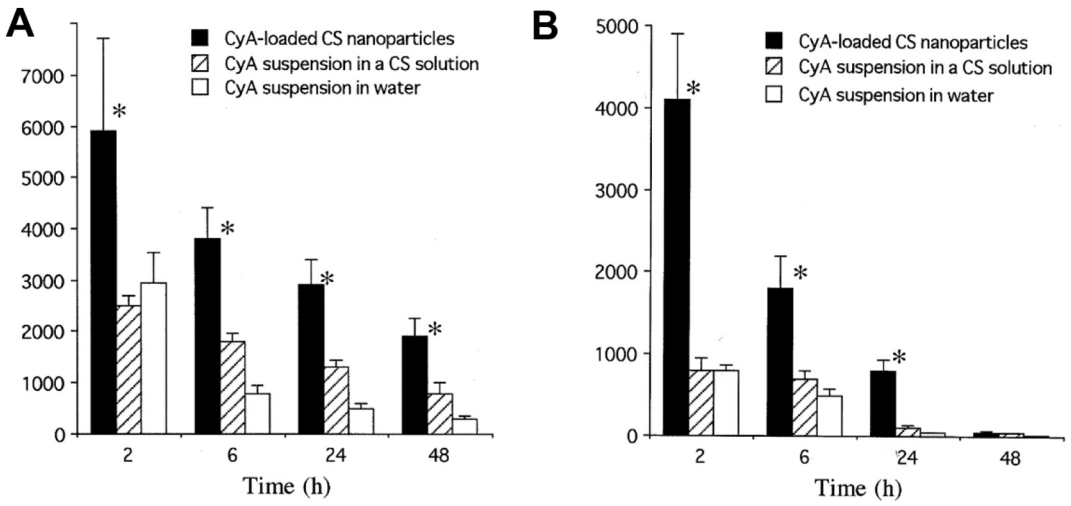

Fig. 4. Cyclosporine A (CyA) concentration in the (A) cornea and (B) conjunctiva after topical administration in rabbits of cyclosporin A-loaded chitosan nanoparticles, cyclosporin A suspension in a chitosan aqueous solution and a cyclosporin A suspension in water. * denotes statistically significant differences, $P < 0.05$. Reproduced with permission of Elsevier from De Campos *et al.* [52].

deacetylation, molecular weight and viscosity. Deacetylation exposes amino groups that make it soluble at pH below 6 (pk_a ~6.5), forming a polycationic polymer that can interact either with anionic crosslinking agents such as tripolyphosphate (TPP) or anionic polymers such as alginate or hyaluronic acid, thus forming polyelectrolyte-based carriers for drug delivery. It is widely recognized that positive charges in the chitosan backbone confer mucoadhesiveness due to electrostatic interactions with the negatively-charged mucosa. However, this mucoadhesive mechanism occurs in the eye to a more limited extent because at ocular physiologic pH (7.4), most amine groups of chitosan are not ionized. Instead, hydroxyl and amine groups of chitosan form hydrogen bonds with mucin [246]. For example, Ibrahim *et al.* demonstrated that chitosan NPs prolonged the effect of brimonidine until 23.2 h post-administration compared to the drug solution which did not exert effect after 7 h [40]. Another feature of chitosan that can be exploited in ocular drug delivery is its ability to transiently open the tight junctions between epithelial cells [247]. This mechanism enhances drug permeation by the paracellular route. In fact, fluorescently-labeled chitosan NPs were detected between the corneal epithelial cells after being administered to the cul-de-sac of rabbits [44]. This result confirmed that chitosan NPs are transported by a paracellular mechanism. Similarly, several studies have proved that this type of carrier can also be internalized by cells through transcellular mechanisms [44,45,248]. De Salamanca *et al.* prepared fluorescein isothiocyanate-BSA (FITC-BSA)-loaded chitosan NPs and evaluated their ability to enter the cell in both *in vitro* and *in vivo* models [45]. First, IOBA-NHC, a cell line derived from normal human conjunctival epithelium, was exposed to the abovementioned fluorescent NPs and examined under confocal laser scanning microscopy using fluorescent FITC-BSA solution as control. Unlike to the evenly distribution of FITC-BSA solution in the cytoplasm, NPs appeared like small fluorescent dots inside the cells from the top to the bottom of the cell monolayer, confirming the intracellular presence of the chitosan NPs [45]. Additionally, FITC-BSA-loaded chitosan NPs were administered to rabbits to further evaluate their eyeballs and eyelids. Results revealed fluorescence throughout the nonnuclear cytoplasm of corneal and conjunctival epithelial cells, to a different degree. Although the uptake mechanism was not fully elucidated and it depends on the cell type, it was suggested that in this case it is not energy dependent [45]. Owing to the preceding advantages, several researcher groups have developed chitosan-based ODDS [40,43,46,48,49,51,52,249]. Remarkably, chitosan NPs showed a better performance than solutions because the nanosytem provides not only a stronger interaction with both the ocular surface and the drug,

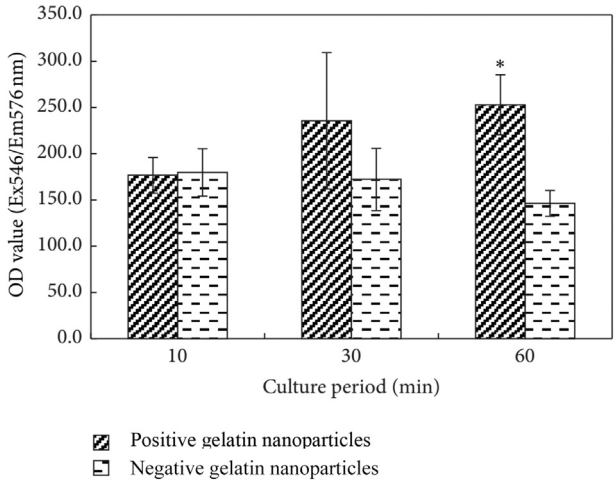

Fig. 5. Fluorescent labeled gelatin nanoparticles uptaken by the human corneal epithelial cells were evaluated by measuring the fluorescence intensity of the cell lysate. n = 6 standard error of mean (SEM), $*P < 0.05$. Adapted with permission of Hindawi from Tseng *et al.* [64].

but also protects the cargo from metabolic degradation [44,52,58]. This superiority was clearly evidenced in an *in vivo* study conducted by De Campos *et al.* in which cyclosporine A was administered as a suspension in water, a suspension in a chitosan aqueous solution and encapsulated within chitosan NPs, to a group of rabbits in five successive instillations [52]. Results showed that animals treated with NPs had significantly higher corneal and conjunctival drug levels than those treated with both suspensions (Fig. 4) [52].

Noteworthy, therapeutic drug concentrations were obtained with chitosan NPs during at least 24 (conjunctiva) and 48 h (cornea) post-administration. On the contrary, cyclosporine A concentration decreased to subtherapeutic levels following 24 h of instilling the drug suspension in chitosan solution [52].

As other polysaccharides, chitosan has functional groups that can be chemically modified to tune its physicochemical properties to ocular delivery [55,57,181,250–252]. For instance, the amine groups of chitosan can be quaternized by exhaustive alkylation to confer a positive charge irrespective of the pH of the medium. Several types of cationic derivatives differing in quaternization degree and molecular weight were tested for ocular delivery [57,250,252]. Although the positive

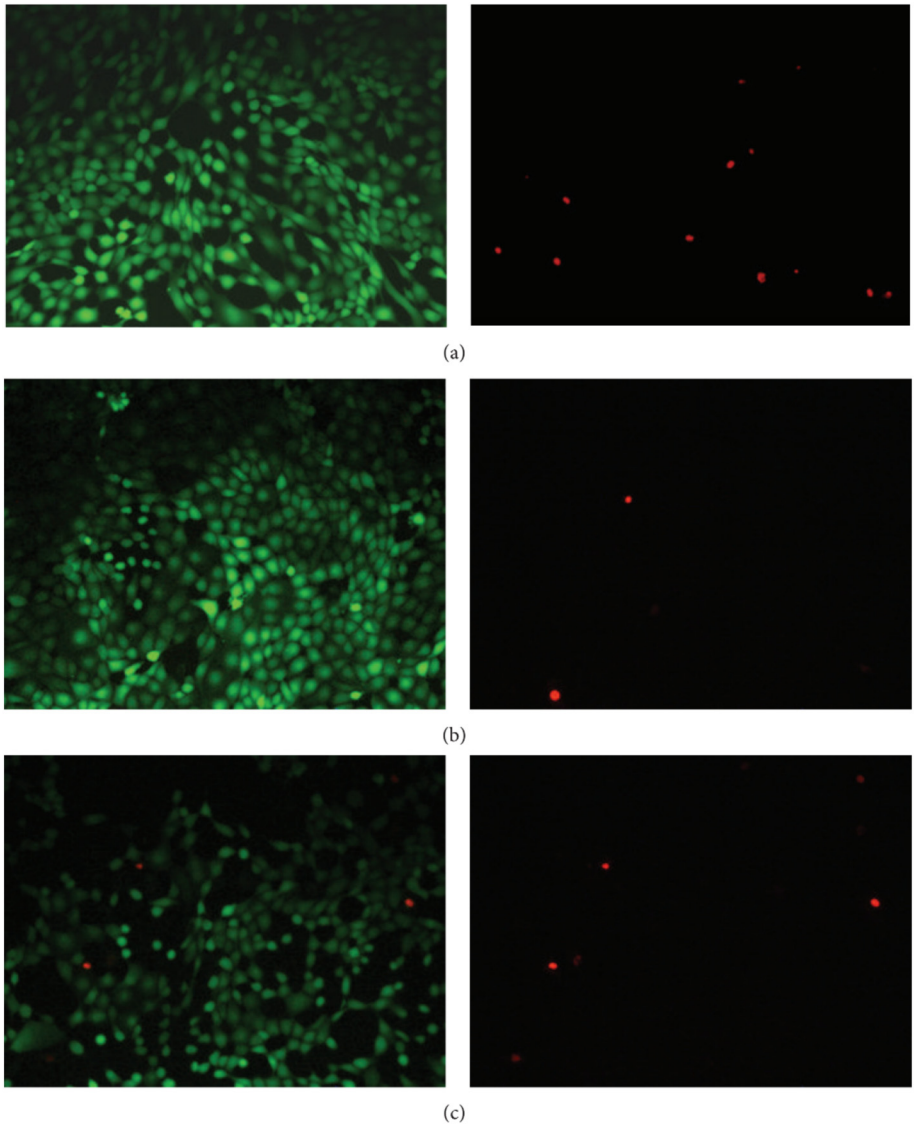

Fig. 6. Fluorescent photomicrographs of human corneal epithelial cells cultured with (a) culture medium, (b) positive gelatin nanoparticles and (c) negative gelatin nanoparticles at a concentration of 0.1 mg/mL for 2 h. The polyanionic dye calcein-AM is well retained within the live cells, which produced intense uniform green fluorescence in the live cells. (Magnifcation: 40x). Reproduced with permission of Hindawi from Tseng *et al.* [64].

charge density plays a critical role in the paracellular absorption-enhancing properties and to a lesser extent in mucoadhesive phenomena (because hydroxyl groups also allow adhesion), it is suggested that there is an optimum value of quaternization above which a greater effectiveness does not take place. This is probably because positive moieties arranged in consecutive units of the chitosan backbone cause not only electrostatic repulsion but also steric hindrance that reduces molecular flexibility of the polymeric chains [253,254]. Another modification widely used is thiolation [255]. This strategy provides better mucoadhesive profiles than the unmodified polymer because thiol groups can form covalent disulfide bonds with the mucus glycoproteins, which are much stronger than non-covalent bonds. The higher mucoadhesion properties of the polymer would enable greater uptake of the encapsulated drug. Zhu *et al.* compared the ocular performance of a FITC solution, FITC-loaded alginate-chitosan NPs and FITC-loaded alginate-thiolated chitosan NPs in rats [181]. Half an hour after instillation of each formulation, the eyes were washed and examined in a stereomicroscope. Then, rats were sacrificed, and the excised corneas were cryosected and observed by confocal laser scanning microscopy. Results of stereomicroscopy showed that although the three kinds of FITC can get into cornea, NPs delivered more cargo than FITC solution. Even, thiolated NPs enabled much better delivery than its non-thiolated counterpart [181]. Other chitosan derivatives were also evaluated for ocular delivery such as *N*-carboxymethylchitosan [251] or galactosylated chitosan [55].

Regarding the safety of the polymer, chitosan is considered GRAS by the US-FDA. In fact, it is approved as a food ingredient [256]. Nevertheless, the ophthalmic toxicity of chitosan carriers should be evaluated. Several reports revealed that chitosan exhibits good ocular tolerance. It is noteworthy that chitosan is biodegraded by lysozyme and other enzymes, which produce oligomers and monomers that can be then cleared [256]. The eye is a rich lysozyme-containing environment, becoming a relevant enzyme in the ocular degradation of chitosan. Its mechanism of action is based on the hydrolysis of the acetylated residues; the greater the degree of deacetylation, the lower the degradation rate [257]. For example, the incubation of chitosan NPs (deacetylation degree of 85%) in a solution of lysozyme at the physiologic concentration in which it is present in human tears fluids (450-1230 μg/mL) [258] did not modify neither their size nor their surface charge, suggesting that the integrity of the NPs is not significantly compromised by the presence of lysozyme in the tears fluid [41,44]. Another advantage related to toxicity is that organic solvents are not

required to produce chitosan carriers, as in the case of hydrophobic synthetic polymers [259]. On the other hand, the toxicity of the NPs depends on its concentration, the target cell and the route of administration. De Campos *et al.* demonstrated that survival of Chang conjunctival cells at 24 h after its incubation with chitosan NPs was high and the viability of the recovered cells was near 100% for all the tested concentrations (0.25-2.00 mg/mL) [44]. However, only cells exposed to chitosan NPs in the range between 0.25 and 1.00 mg/mL exhibited well-preserved morphology and intact cell surface with abundant microvilli and without apparent membrane alterations. Instead, cells exposed to a higher concentration (2 mg/mL) showed small membrane holes and some degree of cell flattening and microvilli loss [44]. Similarly, Wassmer *et al.* assessed the toxicity of chitosan MPs envisioned for subretinal administration at 1 and 10 mg/mL. For this, they primarily evaluated the toxicity *in vitro* by using mouse retinal photoreceptor-derived cells (661W) and then, through an *in vivo* assay by injecting the NPs into the subretinal space of rats [46]. Equally, chitosan NPs at 1 mg/mL did not show signs of *in vitro* cell toxicity, while cytotoxic effects appeared when a higher concentration of 10 mg/mL was used. *In vivo* results were in good agreement with the preceding *in vitro* cell test [46]. These results strongly suggested that regardless of the size of chitosan particles, they could be safely administered at a concentration of up to 1 mg/mL by both topical and subretinal route.

2.2.2. Gelatin

Gelatin is a semisynthetic polypeptide polymer obtained by denaturation of collagen, one of the elements constituting the corneal stroma of the eye. Regarding its amino acid composition, ~13% of the gelatin molecule is positively charged due to lysine and arginine amino acids, ~12% is negatively charged due to the presence of glutamic and aspartic acid, ~11% corresponds to the neutral amino acids leucine, isoleucine, methionine and valine and the rest of the chain to glycine, proline and hydroxyproline [231,260]. Depending on the pre-treatment to which the collagen is subjected prior to the extraction stage, two types of gelatin can be obtained. An acid pre-treatment leads to gelatin type A with an isoelectric point of 7-9, while an alkaline pre-treatment causes the hydrolysis of amide groups of collagen, resulting in a negatively-charged gelatin type B and a reduction of the isoelectric point to 4-5. The molecular weight of gelatin usually ranges between 15,000 and 250,000 g/mol [231].

Gelatin can be used to produce NPs and MPs utilizing different techniques that are thoroughly reviewed elsewhere [261,262] and it was investigated as biomaterial for the production of ODDS [37,62–66,184,185,189,190,199]. Since it is a protein, gelatin becomes an inert vehicle for the delivery of proteins and peptides [63]. The aforementioned versatility in the physicochemical characteristics of the polymer enable the loading of both acidic and basic protein drugs by using gelatin type A and B, respectively. Also, nuclei acid-based molecules like small interfering RNA (siRNA) or plasmid DNA [37,189,190] as well as conventional drugs [62,65,66,185] are efficiently loaded in this type of carriers. On account of the presence of positively-charged amino groups in the structure of gelatin, it can interact with the negatively-charged ocular mucosa, remaining mucoadhered and prolonging the residence time of the encapsulated drug in the absorption site [263–265]. Besides, the presence of arginine–glycine–aspartic acid sequence (RGD motif) confers gelatin cell adhesion properties which reinforces the mucoadhesive potential of the carrier [266]. On the other hand, it is noteworthy that gelatin NPs can be internalized not only by cells like murine bone marrow dendritic cells [267], human fibroblasts [268], human monocytes/macrophages [269] and human lung cancer cells (NCI-H460) [270], but also by HCE cells [64]. This enhances the intracellular delivery of drugs. Interestingly, positive gelatin type A nanoparticles showed greater uptake than their negative gelatin type B counterparts (Fig. 5) [64]. Even so, gelatin surface modification is an outstanding strategy to facilitate the carrier uptake by receptor-mediated endocytosis [262,271]. Up to date, the only derivatization explored in ophthalmic drug delivery is the cationization of gelatin NPs by introducing amine groups to the carboxyl moieties of the polymer [37,189,190]. The positive charge enables not only greater mucoadhesion properties and higher uptake, but also high encapsulation efficiency if the molecule to be loaded is negatively-charged [190,272]. For example, cationized gelatin NPs showed an association efficiency of 93% with enhanced green fluorescent protein plasmid (pEGFP), which is a negatively-charged macromolecule [37]. This system significantly protected the cargo from enzymatic degradation for at least 60 min when naked DNA was completely digested in 5 min.

Moreover, confocal fluorescence microscope images of HCE cells showed effective internalization of the plasmid DNA by NPs, while no uptake was observed with naked DNA [37]. In addition, the paracellular absorption of gelatin NPs was evaluated. For this, the barrier integrity of HCE cell monolayers was followed up by measuring changes in the transepithelial electrical resistance (TEER) of the cells after the incubation with the NPs. Results showed that neither positive nor negative gelatin NPs caused significant differences in the TEER of cells after 96 h, suggesting that gelatin NPs do not increase the drug concentration in the cornea by the intercellular route [64].

Gelatin is another polymer considered GRAS by the US-FDA [273]. It is extensively used in both oral and parenteral products [274]. This provides a large safety window. However, its safety in ocular delivery requires further investigation. Reviewing each key factor in this matter, its biodegradability stands out since the byproducts are not harmful. Moreover, there are techniques that do not involve organic solvents to produce gelatin particles [261,262]. For example, MPs can be obtained by emulsification method using non-toxic materials like vegetable oils. Even, seeing that gelatin is an ampholytic substance, the presence of emulsifiers that are often irritant should be avoided in the formulation. In addition, hybrid polymers can be formed by the ionic gelation method that is organic solvent free. Tseng *et al.* evaluated the cytotoxicity of ~200 nm gelatin NPs on HCE cells by measuring the metabolic activity as an indicator of viability and by fluorescent staining of living/dead cells and subsequent microscopic examination [64]. Results showed that neither positive nor negative NPs were toxic at a concentration of up to 0.5 mg/mL. A large percentage of live cells which emit green fluorescence was observed in the control group as well as in cells treated for 2 h with positive or negative gelatin nanoparticles (Fig. 6) [64]. Only few dead cells which emit red fluorescence were observed [64].

2.2.3. Ethylcellulose

Ethylcellulose is a semisynthetic polymer obtained from cellulose through an alkaline treatment, followed by ethylation with chloroethane. The result is the conversion of some of the hydroxyl groups of glucose units into ethyl ether groups (Fig. 2) [274]. The number of monomers can vary obtaining polymers with different molecular weights. Given its hydrophobic nature, ethylcellulose is widely used for sustaining the release of different drugs. Furthermore, the remaining hydroxyl groups are responsible of its mucoadhesiveness through the formation of H bonds. These features can be exploited for ocular drug delivery purposes and, regardless of the scarce studies available in the literature, results are very promising. For example, the sustained release of dexamethasone was achieved with ethylcellulose NPs [275]. By regulating the polymer concentration, it was possible to fine-tune the degree of release. For example, polymer concentrations of 1%, 2% and 5% w/v released 80%, 60% and 30% of the cargo, respectively, within 24 h (Fig. 7) [275].

Quinteros *et al.* produced acetazolamide-loaded ethylcellulose nanoparticles aimed at optimizing glaucoma treatment [67]. The amount of drug permeated from NPs through isolated corneas was higher than the value obtained with a drug solution. Authors suggested that the mucoadhesive properties of ethylcellulose NPs could promote a close contact with the cornea surface facilitating its penetration. After the instillation of these nanoparticles into rabbit eyes, a greater IOP

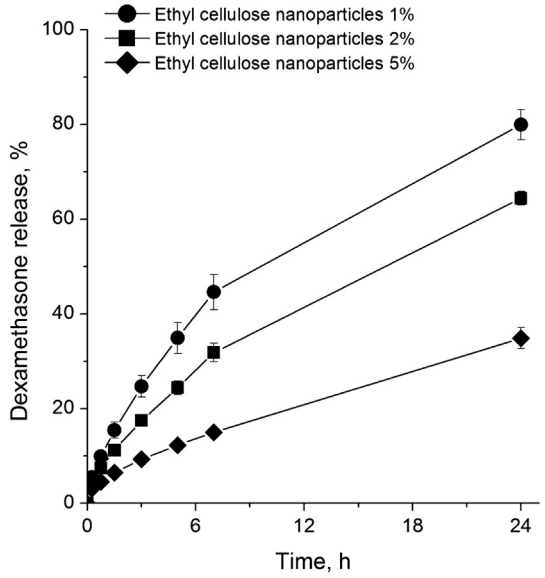

Fig. 7. Effect of ethylcellulose concentration in the nanosuspension on dexamethasone release. Reproduced with permission of Elsevier from Balzus *et al.* [275].

decrease and longer duration of the effect was obtained in normotensive rabbits [67].

Ethylcellulose is a GRAS substance according to the US-FDA and despite it is considered nontoxic, non-allergenic, nonirritant and safe, its ophtlamic biocompatibility must be especially assessed because it is non-biodegradable [274]. The polymer showed good compatibility in ocular inserts [276,277]. However, there are no reports of cytotoxicity by using ethylcellulose particulate systems. On the other hand, it must be considered that since ethylcellulose is hydrophobic, the techniques used to prepare NPs generally involve organic solvents. Therefore, absence of traces in the final product must be ensured by sensitive analytical tools.

2.3. Synthetic polymers

2.3.1. Poly(epsilon-caprolactone)

Poly(epsilon-caprolactone) (PCL) is a synthetic, semicrystalline and aliphatic polyester obtained by the ring-opening polymerization of epsilon-caprolactone (Fig. 2) [278]. The carboxyl terminal group confers a negative charge to the polymer. PCL is hydrolytically degraded by the cleavage of the ester bonds in aqueous media, yielding nontoxic biocompatible degradation products. However, the degradation rate is very slow, from several months to 2–3 years, depending on the molecular weight, degree of crystallinity, and degradation conditions [279–281]. Moreover, PCL is highly permeable to many drugs. Both features support its use in DDS though it should be considered that the release profile not only depends on the characteristics of the polymer but also on the hydrophilicity of the cargo and the particle preparation method. For example, pilocarpine-loaded PCL NPs were prepared as nanospheres and nanocapsules intended for glaucoma treatment [80]. The former is solid with the drug interspersed in the solid core and the later, is a hollow structure that hosts the drug in the core. Both systems exhibited little degradation at 42 days with major degradation after 70 days of incubation in buffer solution of pH 7.4 [80]. However, the hollow skeleton of the nanocapsules led to 3-times greater encapsulation than that allowed by the nanospheres. This was reflected in an enhanced pharmacodynamic profile [80]. Both nanocapsules and nanospheres significantly reduced the IOP values one day after injection into the anterior chamber of rabbit eyes (20 μL, 500 μg/mL). Notwithstanding, the IOP of the animal group treated with pilocarpine-loaded PCL nanospheres gradually returned to the hypertensive baseline after 7 days, while the group treated with pilocarpine-PCL nanocapsules showed suppression of the IOP for 42 days [80]. Beyond nanocapsules and nanospheres, several research groups focused on the development of poly(ethylene glycol) (PEG)-PCL polymeric micelles to improve the water solubility of hydrophobic drugs with promising results [83–86]. For example, diclofenac-loaded PEG-PCL polymeric micelles sustained the release of the cargo [84]. In fact, after 24 h, about 30% of diclofenac remained in the nanocarrier. Furthermore, the amphiphilicity of the nanosystem enhances the penetration of the drug through the lipophilic corneal epithelium and endothelium as well as hydrophilic stroma. Thus, PEG-PCL polymeric micelles increased 17-fold the penetration compared to a drug solution. After instilling diclofenac-loaded PEG-PCL polymeric micelles to rabbits, a twice-fold increment in bioavailability was obtained with respect to the drug solution [84]. In the same way, rapamycin was encapsulated within PEG-PCL micelles and injected to rabbits by the intravitreal route [85]. Fluorescence microscopy revealed that labeled micelles diffused from the vitreous to the retina and localized in the retinal pigment epithelium for at least 14 days. The drug concentration in the retinal tissue was significantly higher with the polymeric micelles than with a drug solution [85]. On the other hand, it has been demonstrated that PCL NPs can be internalized by cells through endocytic pathways enabling a bioavailability increase. For example, 250 nm PCL NPs containing the fluorescent dye rhodamine 6G were applied to rabbit corneas in an *ex vivo* assay [82]. Confocal microscopy images showed the uptake of these NPs with intracellular location at the outer layer of the corneal epithelium [82].

Regarding safety, PCL is a nontoxic and biocompatible polymer widely used in US-FDA approved pharmaceutical products [231]. Nonetheless, the ocular irritation and cytotoxic potential of PCL nanoparticles was assessed to ensure its safe administration by this route. These nanosystems with mean particle sizes between 100 and 300 nm were not irritant by the HET-CAM assay (10 mg/mL) [79]. Similar results were obtained for PEG-PCL polymeric micelles of about 100 nm in rabbits by the Draize test (200 mg/mL) [84]. Additionally, cytotoxicity assays performed in a corneal epithelial cell line (SIRC), retinal pigment epithelium cell line (ARPE-19) and primary human retinal vascular endothelial cells (RVEC), showed time-dependent toxicity of PCL NPs at all doses tested (25-2000 μg/mL) [199,282]. Remarkably, all the concentration range tested resulted in cell viability > 80% for up to 4 days [199,282]. Greater concentrations were tested with PEG-PCL polymeric micelles (0-10 mg/mL) in three types of eye-related cells; HCE cells, human lens epithelial cells (HLEC cells) and retinal pigment epithelial cells (RPE cells). No apparent cytotoxicity up to a concentration of 2 mg/mL was detected, while higher concentrations decreased cell

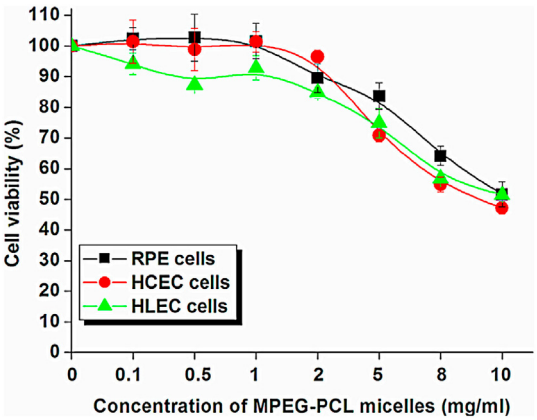

Fig. 8. *In vitro* cytotoxicity of mPEG–PCL micelles against HCEC cells, HLEC cells and RPE cells with concentration in range of 0–10 mg/mL (mean ± SD, n = 6). Reproduced with permission of Elsevier from Xu *et al.* [83].

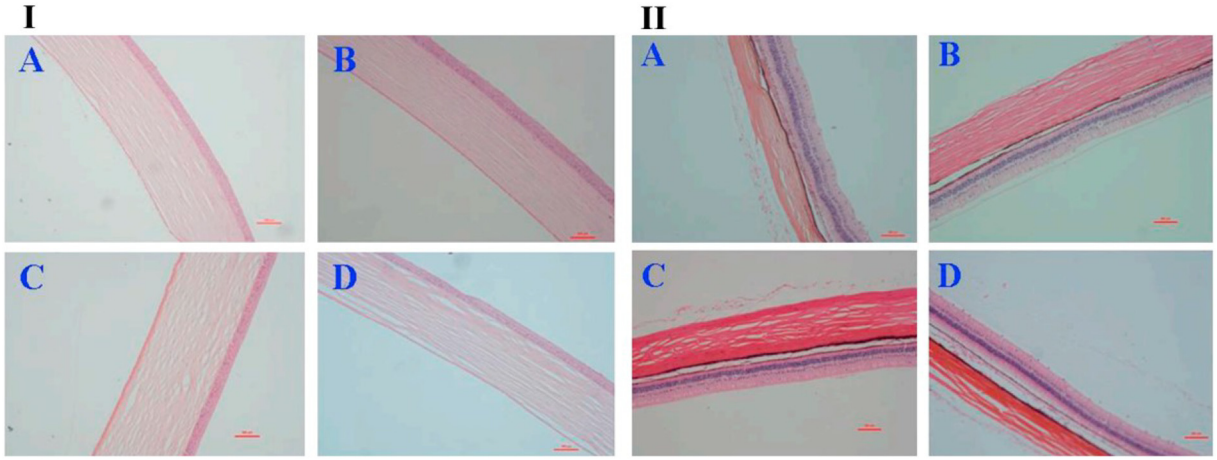

Fig. 9. Hematoxylin and eosin staining of (I) cornea and (II) retina after intracameral and intravitreal injection of mPEG–PCL micelles, respectively. (A) Normal and treated tissue with (B) 100 mg/mL, (C) 150 mg/mL and (D) 200 mg/mL of micelles were evaluated after 3 and 30 days for cornea and retina, respectively. Reproduced with permission of Elsevier from Xu *et al.* [83].

viability (Fig. 8) [83].

In addition, a single intracameral and intravitreal injection of PEG-PCL polymeric micelles (100, 150 and 200 mg/mL) did not cause any changes in microstructure of cornea and retina. These results stated that these nanocarriers did not trigger an immune reaction in these ocular tissues (Fig. 9) [83].

2.3.2. Poly(lactide)

Poly(lactide) (PLA) is a synthetic, hydrophobic, aliphatic polyester obtained by the ring-opening polymerization of lactide (LA) (Fig. 2). The polymer exists in several forms, depending on the precursor used for the synthesis: D-PLA and L-PLA result in PDLA and PLLA, respectively. Both are semicrystalline [283,284]. However, there exist derivatives produced from racemic and meso monomers, namely PDLLA, that are fully amorphous [284]. Depending on the polymerization conditions, different molecular weights and degrees of crystallinity can be obtained. These factors influence the hydrolytic degradation rate that results in lactic acid, a substrate of the tricarboxylic acid cycle resulting in energy, carbon dioxide and water as metabolic products. The greater the degradation rate, the faster the drug release. For example, the anti-cancer drug 5-fluorouracil was loaded in PLA MPs with molecular weight of 4700 and 3400 g/mol and the release sustained for at least 7 days [93]; 70% and 85% of drug was released, respectively. Kimura *et al.* compared the effect of crystallinity on the release profile [87]. By using PLLA and PDLLA of ~5000 g/mol, it was possible to discriminate that the cargo (rhodamine 6GX) was released from PLLA MPs at a slower rate than that from PDLLA counterparts. Results showed that about 12% and 18% of rhodamine 6GX was released after 10 days, while around 20% and 32% after 30 days, respectively [87]. Interestingly, PLA carriers can be internalized by retinal pigment epithelium cell [87,88]. Furthermore, the number of MPs phagocytosed increased with time and the particles remained within the cells for at least 14 days [87]. For this reason, the study of the localization kinetics of PLA NPs after intravitreal administration resulted of utmost importance [92]. In fact, PLA NPs migrate to the retinal layers, tending to accumulate in the retinal pigmented epithelium [92]. Outstandingly, PLA NPs were found within the retinal pigmented epithelium 4 months after a single injection [92]. These findings are very promising to optimize the treatment of diseases affecting the posterior segment of the eye. Moreover, it must be considered that given the hydrophobicity of PLA, poorly-water soluble drugs are encapsulated better than hydrophilic ones. However, appealing to water-in-oil-in-water (w/o/w) or water-in-oil-in-oil (w/o/o) emulsion-solvent-evaporation methods, it is possible to obtain PLA carriers for water soluble drugs, while maintaining the intrinsic property of the polymer to sustain the release. For example, TG-0054 is a highly hydrophilic anti-angiogenic drug with potential to treat choroid neovascularization. This drug was encapsulated within PLA MPs of 7.1 μm in size, which enabled sustained drug release for several months despite the high solubility of TG-0054 [91]. After intravitreal administration to rabbits, significantly greater amounts of drug were detected in retina, as well as in vitreous humor and the choroid-retinal pigmented epithelium [91]. Similar release performances were obtained by injecting PLA microparticles through conjunctival route. Kadam *et al.* produced triamcinolone acetonide-loaded PLA particles of 2 μm in diameter, which sustained the release *in vitro* for 120 days [89]. Following subconjunctival injection, MPs were observed for 2 months at the injection site, enabling sustained release in all intraocular tissues for that period [89]. Conversely, 2 months post-injection, no drug levels were detected in any ocular tissues for PLA NPs or the drug suspension. This is because NPs exhibit higher surface area than MPs, so the drug is released faster. In other words, these results confirmed that MPs are retained for longer time [89]. The use of PLA carriers for ocular drug delivery is supported by its biodegradability and biocompatibility. Additionally, PLA is approved by the US-FDA for human use [284]. The intravitreal injection of PLA MPs (50 μm in size) to rabbits did not cause significant changes in electroretinograms [214]. Furthermore, no histological abnormality was detected in the cornea, lens, retina and choroid.

2.3.3. Poly(lactide-co-glycolide) (PLGA)

PLGA is a synthetic copolymer of PLA and poly(glycolide) (PGA) (Fig. 2). As mentioned before, PLA could be semicrystalline or amorphous. PGA is highly crystalline owing to the lack of the methyl group in the side chain which results in low solubility in different solvents [285]. Thus, it is not used in pure form and combined with PLA. The biodegradability, biocompatibility and sustained release properties of PLGA propelled it to be one of the most extensively explored polymers for sustained ocular drug delivery (Table 2). *In vivo* degradation of PLGA mainly occurs by hydrolysis resulting in lactic and glycolic acids, which further enter to the tricarboxylic acid cycle to be metabolized in energy, carbon dioxide and water [286]. The acid degradation products generated in the interior of the carrier accelerate the degradation process by the so-called autocatalytic process. The greater the degradation rate, the faster the drug release. There exists a wide spectrum of PLGA types with different molecular weight and PLA/PGA weight ratio available on the market, which determines the biodegradation and drug release rate. It is known that polymers with higher molecular weight usually display lower degradation rates because more time is required to degrade longer polymer chains. Thus, intrinsic viscosity of a polymer is directly related to its molecular weight [287]. Araujo *et al.* prepared

flurbiprofen-loaded NPs with PLGA of low (0.32-0.44 dL/g) and high viscosity (0.7-1.1 dL/g) for ophthalmic use and showed that the latter releases the drug at a slower rate than NPs produced with low viscosity PLGA [114].

However, it must be considered that the molecular weight affects the degree of crystallinity. Usually, polymers of low molecular weight crystallize faster than high molecular ones [288,289]. Conversely, owing to the contribution of polymer chain edges to amorphousness, high molecular weight polymers usually result in higher degree of crystallinity than low molecular weight counterparts and thus, slower release. It is possible that in some cases PLGA nanoparticles of low molecular weight release the drug at a slower rate than the high molecular weight counterpart [289]. Regarding the PLA to PGA ratio in the copolymer, it is noteworthy that increasing the percentage of LA leads to a slower degradation rate. This is because methyl groups increase the hydrophobicity of the polymer chain and hence it absorbs less water and degrades more slowly [290]. In this context, Ogura *et al.* produced MPs of PLGA 50:50 and 75:25 molar ratio of DL-PLA:PGA with the same molecular weight (MW = 5000 g/mol) to be administered to rabbits by the subretinal route [88]. The *in vitro* release profile was more sustained with PLGA 75:25 MPs than with the more hydrophilic derivative; approximately 18% and 50% of drug (rhodamine 6GX) was released after 10 days with PLGA 75:25 and 50:50 MPs, respectively. These values increased to 26% and 90% at 20 days and 65% and 98% at 30 days [88]. Despite it is possible to select the suitable polymeric features to adjust the release profile, it should be stressed that it also depends on the nature of the drug, the drug load, the size of the carrier and even the preparation method [129]. Thereby, hydrophilic drugs are released faster than hydrophobic ones and particles with higher drug loading lead to more significant burst release and smaller particles exhibit higher surface area leading to higher degradation of the matrix with consequently faster release. Besides, it must be taken into account that PLGA carriers can encapsulate both hydrophilic and hydrophobic drugs, although owing to affinity issues, hydrophilic ones exhibit lower encapsulation efficiency. Moreover, when the cargo is a protein, the harsh conditions required to prepare PLGA NPs, especially the double emulsion solvent evaporation method that employs organic solvents results in protein aggregation and/or denaturation and subsequent inactivation. Equally, the acid environment generated during the degradation of the particle can also denature the protein. This is why the addition of stabilizers to the formulation is required to overcome these limitations. For example, Varshochian *et al.* prepared PLGA NPs containing bevacizumab, an antibody intended for ocular neovascularization treatment [207]. To preserve the bioactivity of the cargo, a proper concentration of albumin was added to the NPs. Unlike other polymers, PLGA is not mucoadhesive. Thus, PLGA particulate systems are administered by intravitreal or subconjunctivally routes rather than being applied topically. Dexamethasone sodium phosphate-loaded NPs prepared with PLGA 50:50 molar ratio of DL-lactide:glycolide, molecular weight ~3.2 kg/mol, acid terminated, sustained drug release over 15 days *in vitro* [133]. After being administered to the subconjunctival tissue of rats, NPs sustained ocular drug levels for at least 7 days and prevented corneal allograft rejection over the entire 9-week study when administered weekly. Conversely, animals that received weekly injections of either placebo NPs, saline, or a drug solution evidenced corneal graft rejection with severe corneal edema, neovascularization and opacity that occurred in less than 4 weeks [133]. Similarly, celecoxib-loaded PLGA MPs (PLGA 85:15, intrinsic viscosity = 0.67 dL/g) sustained the release not only *in vitro* but also *in vivo* for 60 days after being subconjunctivally administered to rats [110]. The consequence was the inhibition of diabetes-induced elevations in prostaglandin E2 (PGE2) and vascular endothelial growth factor (VEGF), and blood–retinal barrier leakage. In contrast, there was no significant effect neither with the placebo-treated eyes nor the contralateral eyes in celecoxib-PLGA microparticle-treated rats [110]. On the other hand, PLGA 75:25 of molecular weight 15 kg/mol was used to microencapsulate cyclosporine A as a possible treatment for uveitis [124]. The intravitreal injection of drug-loaded MPs to rabbits maintained therapeutic cyclosporine A concentrations for at least 65 days in choroid-retina and iris-ciliary body, the tissues involved in the disease [124]. Other encouraging results were obtained with PLGA carriers administered directly to the vitreous or conjunctiva (Table 2). As mentioned above, PLGA is not mucoadhesive though it can be retained longer in the mucosa compared to conventional aqueous eye drop solutions, which are rapidly cleared from corneal and conjunctival surfaces. This was clearly evidenced in a work conducted by Gupta *et al.* in which sparfloxacin-loaded PLGA NPs (PLGA 50:50, intrinsic viscosity = 0.2 dL/g) were administered to rabbits detecting a longer residence time at the corneal surface with respect to the marketed eye drops [32]. This was assessed by gamma scintigraphy using the radiolabeled drug (Fig. 10). The marketed formulation was rapidly cleared from the cornea reaching the systemic circulation, and being detected especially in kidneys and bladder 6 h after instillation (Fig. 10A).

Conversely, PLGA NPs were retained at the corneal surface and remaining undetected in the systemic circulation even after 6 h (Fig. 10B). Complementary studies of radioactivity count with respect to time on the cornea supported the greater retention of NPs with respect to the marketed formulation (Fig. 10C). On the other hand, an interesting feature of PLGA particles is that they can be internalized by cells increasing the bioavailability [36,105,109,291,292]. The uptake occurs by endocytosis, mainly by clathrin- and caveolin-mediated pathways [291]. There is a correlation between the particle size and its uptake. For example, Qaddoumi *et al.* showed a higher uptake of 100

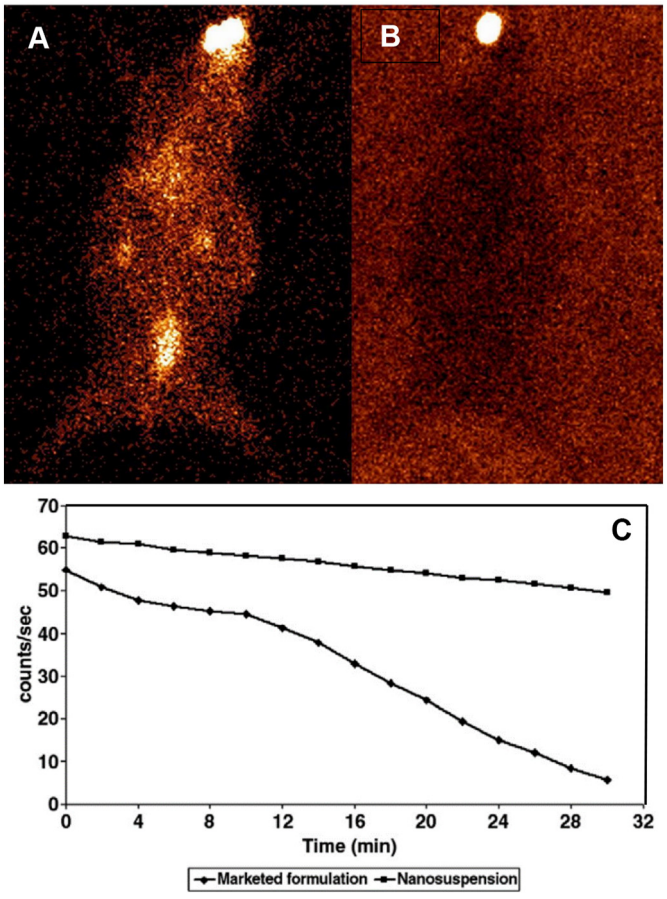

Fig. 10. *In vivo* ocular retention study using gamma scintigraphy. (A) Whole-body images after 6 hours of administration of marketed formulation and (B) sparfloxacin-PLGA nanoparticles. (C) Time-activity curves showing precorneal drainage. Reproduced with permission of Elsevier from Gupta *et al.* [32].

nm than 800 nm and 10 μm particles in rabbit conjunctival epithelial cells [36].

Regarding safety issues, PLGA is approved by the US-FDA in different DDSs [274,293]. It is biodegradable and the degradation products are non-toxic. However, the formation of acidic by-products (lactic and glycolic acid) during degradation increases the local acidity that may lead to irritation of the surrounding tissue. In this context, ocular irritation tests are needed to ensure the safety of these delivery platforms. Results showed that PLGA NPs with varying ratio of LA:GA and molecular weight, at a concentration and size of up to 9.5 mg/mL and 350 nm, respectively, were non-irritant by the HET-CAM study [114,115,118,119,205,292]. Higher concentrations and sizes were not evaluated. Warsi *et al.* assessed the irritating potential of PLGA NPs in a non-invasive *in vivo* study by measuring the temperature of rabbit eyes after instilling the nanosuspension using an infrared camera [117]. An increase in temperature of the corneal surface by 5°C was considered as a sign of inflammation in the eyes. Results showed that PLGA NPs elevated the eye temperature by approximately 2°C, indicating that they were not irritant [117]. On the other hand, the corneal hydration is useful as an indicator of toxicity, considering that a hydration level that is 3-7% units above the normal value of the healthy cornea hydration (76-80%) reveals damage of the epithelium or endothelium. Remarkably, PLGA NPs in the concentration and size mentioned above (9.5 mg/mL and 350 nm, respectively) did not damage the cornea [111,112,118]. In addition, histopathology studies of excised goat cornea after its incubation with PLGA NPs demonstrated a normal morphology of corneal cells [113,116,117]. The *in vivo* Draize test is still used to evaluate ocular irritation because it enables to analyze effects not only in conjunctiva but also in eyelids, cornea, iris and the anterior chamber. These results are in agreement with those obtained by HET-CAM studies [111,116,118,121,203,292]. Beyond the potential irritation provoked by PLGA particles, its cytotoxicity should also be considered. PLGA NPs of around 130 nm did not display any significant cytotoxicity in a concentration of up to 7.5 mg/mL after 24 and 48 h in a HCE cell line [106,107]. Similarly, PLGA nanoparticles of 350 nm were nontoxic in a Y-79 human retinoblastoma cell line [111]. MPs of 6.83 μm showed no statistically significant death neither on Schwann cell derived cell line RSC96 (ATCC CRL-2765) nor on fibroblast-like cell line MC3T3-E1 Subclone 4 (ATCC CRL-2593), skeletal muscle cell line L8 (ATCC CRL-1769) and chondrocyte cell line (ATDC5) [104]. Although it is reported that PLGA NPs caused a low percentage of L929 (mouse fibroblast) cell death, authors explained that it was probably due to the presence of organic solvent residues in the nanosuspension [109]. However, as we explained before, cytotoxicity assays should be performed in the target cell to be reliable toward *in vivo* studies. In a similar way, it is relevant to evaluate eye biocompatibility and safety of PLGA particles after being administered by the intended administration route. Thus, after the subconjunctival injection of PLGA NPs (size of 200 nm, dose of 1 mg) to rats, mild inflammation in conjunctiva near the injection site was observed after 2 days, while a recovery was observed at days 7 and 14 [133]. Furthermore, no inflammation in the cornea at any time point was detected. Similar profiles were obtained with the control (saline injection) [133]. Intravitreal injection of 20 μg of PLGA MPs of 1-2 μm did not cause significant microglia reaction, while 200 μg of MPs did [127]. Conversely, rats treated with a dose of 500 μg of 27 μm MPs injected intravitreally did not show a reduced mean number of retinal ganglion cells with respect to the control [294]. Furthermore, Rong *et al.* performed a complete safety evaluation of MPs composed of PLGA and PLA with sizes ranging 40 to 100 μm through injection in rabbits [214]. No preclinical evidence of ocular changes in animals treated with doses of 2.5, 5 and 10 mg of PLGA were observed. In addition, terminal deoxynucleotidyl transferase-mediated dUTP nick end (TUNEL) labeling evidenced no apoptotic cells in retina after the administration of the three doses of PLGA MPs. Moreover, there was no increase in glial fibrillary acidic protein expression, which is considered a factor contributing to neuronal death and the retinal microstructure and ultrastructure remained intact. These results supported that no inflammation or cell toxicity in the retina was triggered by the MPs or their degradation products. Likewise, electroretinograms were normal in eyes treated with any of the doses tested, which evidences that these microparticles in the vitreous did not cause retinal functional damages [214].

2.3.4. Poly(ortho ester)s

Poly(orthoester)s (POEs) are hydrophobic polymers with hydrolytically labile orthoester bonds. There are four generations of POE which are extensively reviewed elsewhere (Fig. 2) [295]. However, only POE type III and IV were studied for ocular application and the latter for ODDS. This is because POE type III is synthesized by a multistep method, poorly reproducible and difficult to industrially scale up. Moreover, the resulting product is semi-solid what precludes its use to prepare NPs [296].

The hydrophobicity of POE seems appealing to sustain drug release because it enables a zero-order release kinetics and like other polymers by varying its molecular weight, the release kinetics can be conveniently adjusted. For example, epinephrine-loaded NPs prepared with POE of 5 and 22 kg/mol released 29.5% and 20.4% of drug within 14 weeks and following a zero-order profile [136]. The group of Jablonski demonstrated that POE NPs were not internalized by Muller or HEK-293 cells [136,137].

Regarding ocular safety, POE IV polymers were evaluated through subconjunctival, intracameral, intravitreal and suprachoroidal administration routes, exhibiting good biocompatibility [296]. Progressing toward the evaluation of POE NPs, Palamoor *et al.* tested the cytotoxicity of delivery systems prepared with POE of two different molecular weights (5 and 22 kg/mol) on HEK-293 cells [136]. As shown in Fig. 11, NPs with size of 290 nm showed low cytotoxicity exhibiting cell viability between 84% and 100%, except for 1 mg/mL NPs which

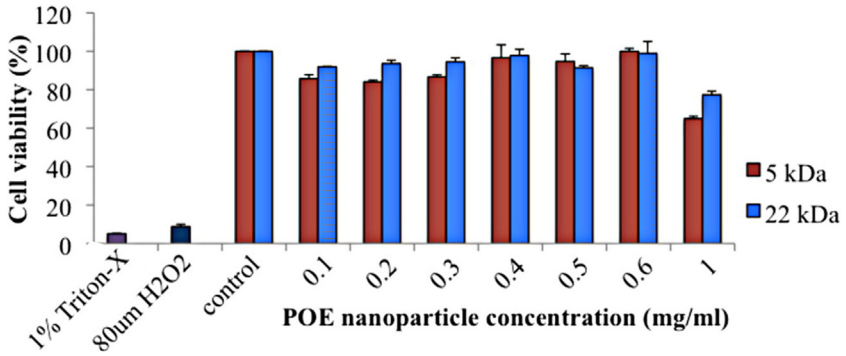

Fig. 11. Cytotoxicity of 290 nm-sized nanoparticles of 5 and 22 kDa molecular weight POE. Positive controls, 1% Triton-X 100, and 80 μm H_2O_2. Data shown as mean ± standard error (n = 3). Reproduced with permission of American Chemical Society from Palamoor *et al.* [136].

showed 65% and 77% of cell viability [136]. Moreover, ophthalmic tolerance of POE forming nanoparticles in two different concentrations (1.5 and 10 mg/mL) were assessed in rabbits and mice after their intravitreal injection. Both levels resulted to be nontoxic as determined by clinical and functional examination [138].

2.3.5. Poly(acrylate)s and poly(methacrylate)s

There is a wide range of synthetic polymers based on acrylates and methacrylates that have been studied for ocular drug delivery. Among them, copolymers derived from esters of acrylic and methacrylic acid whose brand name is Eudragit®, mainly commercialized by Evonik®, are a well-known coating material for conventional tablets since 1954 [297]. However, its use as matrix formers of NPs and MPs is growing due to the great versatility offered by commercially available products. Depending on the functional groups in the side-chain of the polymer its dissolution profile changes. Thus, there are four major families available (Fig. 2). Cationic Eudragit® E is an amino alkyl methacrylate copolymer. There are three subtypes; E100 available as granules, E 12.5 as an organic solution and E PO as a powder. All of them are soluble at pH < 5, and swellable and permeable at pH > 5. On the other hand, anionic Eudragit® L, S and FS are methacrylic acid copolymers. L 100-55 and L 30 D-55 subtypes, available as powder and aqueous dispersion, respectively, dissolve above pH 5.5. Eudragit® L 100 (powder) and L 12.5 (organic solution) are soluble at pH > 6. Finally, Eudragit® S 100 (powder), S 12.5 (organic solution), FS 100 (powder) and FS 30 D (aqueous dispersion) are soluble at pH > 7. The third family corresponds to Eudragit® RL and RS. They are ammonioalkyl methacrylate copolymers. Both of them are available as granules (RL 100 and RS 100), powder (RL PO and RS PO), organic solution (RL 12.5 and RS 12.5) and aqueous suspension (RL 30 D and RS 30 D). They are insoluble at physiological pH values and show a pH-independent swelling profile. The difference between them lies in the amount of quaternary ammonium groups being 4.5-6.8% and 8.8-12.0% for RS and RL, respectively. Therefore, the former exhibits low water permeability, while the latter shows high permeability. Thus, it is possible to customize the release profile by combining RS and RL subtypes in different ratios. Finally, the family of neutral Eudragit® NE are methacrylic ester copolymers, which are insoluble at physiologic pH, with pH-independent swelling and low permeability. Unlike Eudragit® RS, the NE subtype is highly flexible because it contains nonoxynol 100 as surfactant [298,299]. Despite the vast range of available Eudragit® prototypes, RS and RL subtypes were the only ones explored for ocular nano-drug delivery (Table 2). Probably, because these polymers enable a better control of the drug release kinetics. Moreover, they display pH-independent positive charge so, they can interact with negatively charged mucosa, conjunctiva and cornea, prolonging the residence time of the encapsulated drug [149,155]. Both features lead to a greater drug permeation. For example, terbinafine hydrochloride-loaded Eudragit® RS100 NPs instilled to rabbits prolonged the mean residence time of the drug from 2.2 h obtained with an oily drug solution to 4.8 h [149]. In addition, t_{max} was obtained later (1 and 2 h for oily drug solution and NPs, respectively). Thus, the above mentioned physicochemical characteristics of Eudragit® RS and RL are translated as better pharmacokinetic [27,148,149,152,156,200,209] and pharmacodynamic profiles [24,25,67,147,150,151,153,154,156,159]. Despite some reports that suggest possible corneal uptake of Eudragit® RS and RL NPs [24,146,148,154], until now there is no conclusive evidence to confirm this. Kesarla *et al.* exposed goat eye to a suspension of Eudragit® RL 100 NPs containing fluorescein dye for 12 h [145]. Then, eyes were examined by confocal microscopy, where it was possible to observe fluorescence. However, it is indispensable at least to repeat the assay using a fluorescein solution as control [145]. Morsi *et al.* encapsulated ketorolac tromethamine within Eudragit® RL NPs that were instilled to rabbits, using commercial eye drops Acular® as control [148]. A 1.4-fold increase in C_{max} was achieved by nanosuspension in a longer time (2 h) compared to eye drops (1 h). Authors argued that the C_{max} increase was because drug-loaded NPs could penetrate the corneal epithelial cells by endocytosis and that the longer t_{max} stemmed from the longer time required for NP uptake and drug release. However, the increase of C_{max} and t_{max} could be due the electrostatic retention of Eudragit® RL 100 NPs in the precorneal surface. Therefore, additional studies are needed to confirm any of these hypotheses [148]. Eudragit® NE could also represent an interesting platform for ODDS. However, Cortesi *et al.* demonstrated that acyclovir is released very fast when Eudragit® NE is part of the carrier because the surfactant contained in the commercial polymeric aqueous dispersion acts as dissolution enhancer [143].

Aiming to tailor the structure of polymers and thus, to suit pharmaceutical demands, some chemically modified Eudragit® have been developed. Major modifications were focused on the anionic prototypes with the objective of conferring them mucoadhesive properties. Thus, thiolated Eudragit® was prepared by conjugation of L-cysteine to the carboxylic acid moieties via amide bond formation [300–303]. Thiol groups form covalent disulfide bonds with mucus that are stronger than inter-molecular forces such as electrostatic bonds, H bonds and van der Waals bonds. Eudragit® E derivatives were chemically modified by partially quaternization of amine pendant groups [304]. The result is a polymer with pH-independent positive charges, which would confer not only mucoadhesion properties to Eudragit® E but also an improvement of the solubility under neutral to basic medium, as it is typical in the eye. It is important to stress that regardless of a similar composition to other water-insoluble derivatives, they display higher ratio between positively-charged and hydrophilic repeating units (that favor aqueous solubilization) and hydrophobic ones (that preclude the increased the solubility of these derivatives). This is a good example of the great synthetic versatility of polymers and the reason why they are key players in the field of drug delivery. Besides, the quaternized Eudragit® E increased the paracellular permeation at pH 7.4 [304]. These few but promising reports serve as a starting point to be evaluated by an ocular route.

Regarding safety issues, Eudragit® copolymers are approved by regulatory agencies for use in non-parenteral pharmaceutical products. In addition, some poly(methacrylate)s are used in biostable medical implants such as bone cement and intraocular lenses [231]. Its synthetic nature ensures higher quality levels and lower contamination with undesired (e.g., immunogenic) residues than those obtained with natural polymers. Several reports established that Eudragit® RS and RL NPs are non-irritant according to the above described Draize test in a concentration range between 0.1 and 8 mg/mL [23,25,27,153,158]. Only 8 mg/mL of NPs instilled after a storage of 4 months showed a negligible conjunctival hyperemia 10 min after the treatment, while no irritation signs were observed after 6 and up to 24 h. Both copolymers exhibit the same degree of tolerability, despite the different amount of ammonium groups [23]. Histopathological studies of eyes exposed to this concentration of Eudragit® RL 100 NPs also showed no irritation [145]. Similarly, Katzer *et al.* classified Eudragit® NPs as non-irritant by the HET-CAM assay [79]. The toxicity of these carriers was determined by MTT assay in HEC cells and the results were compared to those obtained with a gatifloxacin solution and gatifloxacin-loaded Eudragit® NPs. Acceptable cytotoxicity results were obtained in a range of polymeric concentrations of 0.5-5.4 mg/mL without concentration-dependent trends. These values corresponded to drug concentrations of 0.1-1.0 mg/mL when considering a mean drug loading of 46%. Empty NPs exhibited lowest toxicity than a drug solution and drug-loaded NPs [144]. However, given its non-biodegradability, nanocarriers and microcarriers produced with Eudragit® require rigorous studies of toxicokinetics and acute and chronic ocular toxicity. On the other hand, the safety of NP production methods should be also considered. Common techniques used to prepare Eudragit® carriers such as nanoprecipitation or double emulsion techniques involve organic solvents, though some technologies enable complete removal of solvent residues. For instance, Cortesi *et al.* prepared MPs through the spray-drying

technique injecting a water suspension of Eudragit® (RL, RS and NE) in the drying chamber of the apparatus until total evaporation of the solvent [143]. Since the complete removal of the organic solvent is ensured, there would be no major problems in using the above-mentioned techniques.

Beyond Eudragit® family, other poly(acrylate)s have been explored as particulate ODDS. Poly(alkyl cyanoacrylate) (PACA) is one of the first polymers investigated for this purpose owing to its excellent bioadhesive properties [35,160,162,163,165,167,305]. In fact, it is used as surgical glue for the closure of skin wounds [306]. As mucoadhesive carriers, Wood *et al.* demonstrated that poly-hexyl-2-cyanoacrylate NPs remained longer in tears than drug solutions [163]. Similarly, γ-scintigraphy studies enabled to determine that In-111-labeled poly-butyl-2-cyanoacrylate NPs drained at a significantly slower rate than isotope solutions [162]. On the other hand, the absorption of PACA NPs was investigated. The uptake was demonstrated in different cells like prostate cancer cell line (PC3), rat brain endothelial cell line (RBE4) and Vero cells [307,308]. Even though none of these cell lines belong to the eye, these findings encourage their study in ophthalmic delivery. Zimmer *et al.* assessed the transport of poly-butyl-2-cyanoacrylate NPs labelled with fluorescent dyes in rabbit cornea and conjunctiva by laser scanning confocal microscopy [160]. Fluorescent particles were observed inside the cells as granules or vesicles, while penetration through tight junctions was not observed. On the contrary, a solution of the dye did not enter into the cells. These findings suggested that NPs are endocytosed by the cells. However, lysis of the cell wall by NP metabolic degradation products is another explanation suggested by the authors [160]. The abovementioned leads to look carefully at the potential toxicity of PACA. This family of synthetic polymers is biodegradable. The main mechanism of degradation relies on esterases that catalyze the hydrolysis of the ester bond, resulting in the corresponding alkyl alcohol and poly(cyanoacrylic acid) [309]. This is why the toxicity of PACA is related to the length of the alkyl chain. The longer the alkyl rest, the slower the hydrolysis and the lower the toxicity [310,311]. Few studies about the toxicity of PACA NPs in ocular tissues are available. The intravitreal injection of poly(ethyl-cyanoacrylate) NPs with sizes ranging between 250 and 810 nm in rabbit eyes showed some toxicity, reflected in lens opacification [164]. PEG-coated poly(ethyl-2-cyanoacrylate) NPs with a mean size of 190 nm were well tolerated, according to the Draize test [161]. Poly(acrylic acid) NPs have also been evaluated for ocular drug delivery exhibiting controlled drug release and adhesive properties. Furthermore, this carrier with sizes in the range of 50 nm was found to be non-toxic in an *ex vivo* test where HCE cells retained their viability after being incubated with poly(acrylic) acid NPs [168,169,312,313].

3. Hybrid polymer carriers for ophthalmic drug delivery

As we extensively discussed in the previous section, there is a wide range of polymers for ODDS. Even though drug-loaded colloidal systems show better performance than drug solutions, there are still some drawbacks that should be overcome. Hydrophilic polymers such as alginate, chitosan or gelatin are ideal to encapsulate water-soluble drugs but fail to sustain the release in the aqueous biological milieu. Conversely, hydrophobic polymers such as PCL, PLA, PLGA, and Eudragit® enable not only to tune the release profile but also to diminish or eliminate the burst effect, at the expense of a low efficiency of encapsulation of water-soluble drugs. In addition, despite the good biocompatibility of natural polymers, the synthetic counterparts ensure superior production quality. In this scenario, the combination of polymers from different sources and displaying a broad spectrum of properties in the same ODDS has been proposed. For example, the combination of alginate and chitosan was extensively studied for ocular drug delivery [175–180]. These polymers form polyelectrolyte complexes by virtue of the electrostatic interactions between the carboxyl groups of alginate and the amine groups of chitosan. Beyond the advantages of each polymer, its combination increases the mechanical stability of the system, decreases the permeability of drugs and sustains the release of the cargo more efficiently than either alginate or chitosan alone. Moreover, the coating of hybrid particles improves the performance even more. For example, chitosan-coated sodium alginate–chitosan NPs loaded with 5-fluorouracil resulted in significantly higher drug concentration in both aqueous and vitreous humor after instillation in rabbit eye as compared to sodium alginate–chitosan NPs and drug solution (Fig. 12) [178]. These systems were non-irritant according the Draize test [178].

Other combinations such as chitosan and gelatin [184,185] or chitosan and albumin [182,183] were developed with the same purpose of improving carrier stability. Furthermore, binding two hydrophilic polymers with opposite charges enables to produce carriers without using organic solvents [186–188].

PLA, PLGA, PCL are efficient to control the release of the cargo, though they lack mucoadhesiveness. In this context, the development of a controlled release core containing drug and subsequently coated with a mucoadhesive polymer is a suitable strategy to avoid the rapid ocular clearance of the carrier, while maintaining a sustained release over time [198,199,201,203,205,206,314]. Mahaling *et al.* showed the increase in the degree of mucoadhesion of PCL NPs after being coated with several mucoadhesive polymers such as chitosan, gelatin and Pluronic® F68 (Fig. 13) [199].

Salama *et al.* produced chitosan-coated fluocinolone acetonide-

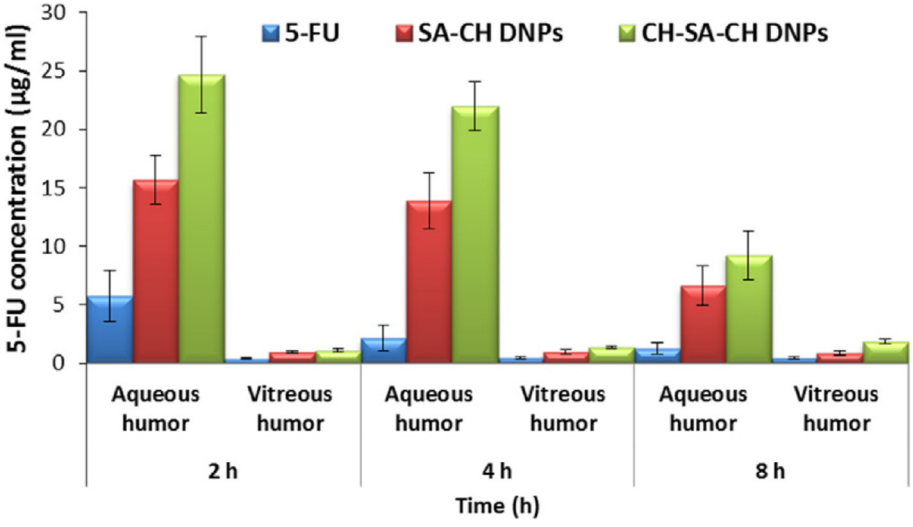

Fig. 12. Comparison of 5-fluorouracil (5-FU) level in aqueous humor and vitreous humor of rabbit eye at different time intervals when administered in free form (5-FU) or encapsulated within sodium alginate-chitosan (SA-CH DNPs) and chitosan-coated sodium alginate-chitosan (CH-SA-CH DNPs) nanoparticles. Reproduced with permission of Elsevier from Nagarwal *et al.* [178].

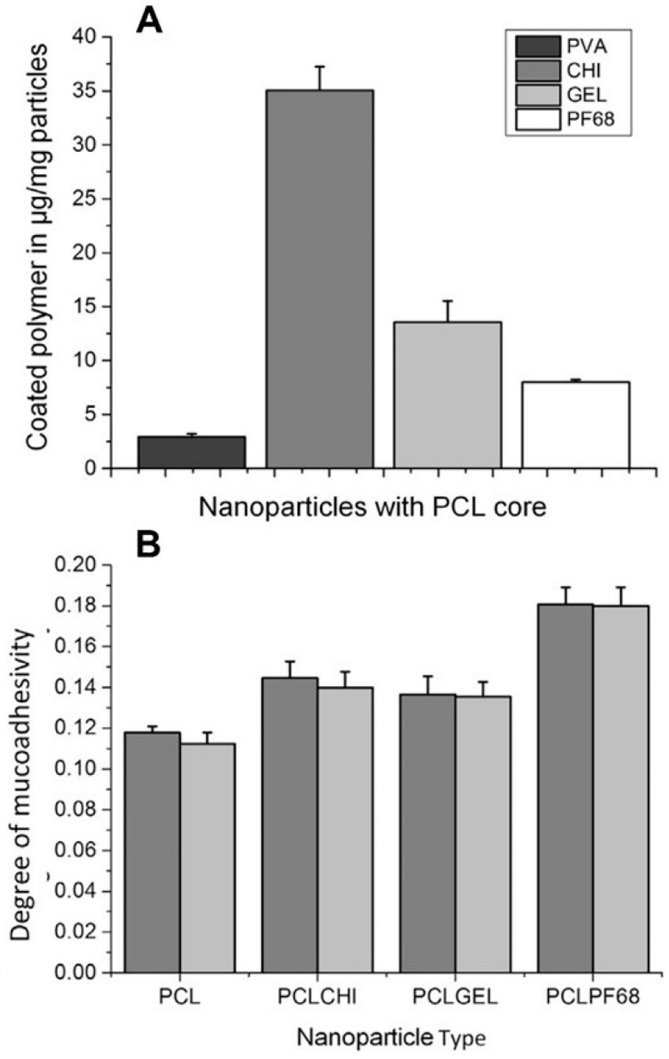

Fig. 13. (A) Quantification of amount of polymer coated on PCL nanoparticles, (B) Degree of mucoadhesivity. (n = 4). Reproduced with permission of Elsevier from Mahaling *et al.* [199].

loaded PLGA NPs [203]. The mucoadhesiveness of this system was demonstrated by the mucin suspension method. Results showed that zeta-potential and particle size values changed from +17.8 to +1.9 mV and from 779.5 to 1302.5 nm, respectively, after incubation with mucin solution. These findings were not observed in the case of uncoated NPs due to their lack of mucoadhesiveness. Hence, *in vivo* studies revealed prolonged effect with chitosan-coated PLGA NPs: the drug level was undetectable after 4 h with uncoated PLGA NPs, while chitosan-coated NPs showed detectable drug levels up to 10 h [203]. Consequently, the $MRT_{0\text{-inf}}$ was 1.34 and 2.88 h for uncoated and mucoadhesive coated NPs, respectively. Thereby, the C_{max} and $AUC_{0\text{-inf}}$ increased from 3.98 to 8.72 ng/mL and from 4.63 to 24.21 ng/mL/h, respectively [203]. This higher absorption with chitosan-coated NPs was probably due to a prolonged residence of drug in the absorption site as well as a higher cellular uptake [314]. Similarly, Calvo *et al.* demonstrated that chitosan coated indomethacin-loaded PCL nanoparticles increased the bioavailability in cornea and aqueous humor when compared to uncoated ones (Table 3) [198].

PLGA NPs enabled a 3.2- and 4.3-fold bioavailability increase, respectively, with respect to a drug solution in cornea and aqueous humor. However, chitosan-coated NPs led to an even better performance [198]. It is worth mentioning that a coating is not the only strategy to confer mucoadhesiveness [211,275]. Choy *et al.* entangled PLGA as sustained core material, and PEG as mucoadhesiveness promoter, forming a hybrid matrix which adhered better to the mucous membrane than conventional (PEG-free) PLGA NPs [211]. As seen in Fig. 14A, 29% of uncoated carriers remained adhered, while 53% of coated ones were retained. Confocal images were in good agreement with these findings (Fig. 14B) [211].

Aksungur *et al.* prepared cyclosporine A-loaded NPs using either PLGA or a mixture of PLGA with Eudragit® RL or Carbopol®-coated PLGA NPs [109]; Carbopol® are carbomers, a synthetic type of polymers based on acrylic acid that are crosslinked with either allyl sucrose or allyl ethers of pentaerythritol. They have high molecular weight with high amount of carboxylic acid groups that can form hydrogen bonds with mucus [231]. Results indicated that the uptake of drug-loaded NPs increased after coating them with the mucoadhesive Carbopol® (Fig. 15) [109]. It is well-known that the more intimate the contact between NPs and cell surface, the greater is the likelihood of carrier uptake. Hence, the highest internalization was achieved with PLGA/Eudragit® NPs, most probably because the positively-charged particle surface facilitated electrostatic interaction with the negatively charged L929 cells membrane [109].

These NPs were smaller than the coated ones, favoring even more the cellular uptake [109]. These *in vitro* results were reflected in an enhanced pharmacokinetic profile with $AUC_{0\rightarrow 24h}$ values of 490.4, 776.6 and 972.6 ng.h/g tear for drug-loaded PLGA NPs, Carbopol®-coated drug-loaded PLGA NPs and drug-loaded PLA/Eudragit® NPs, respectively [109]. Another popular approach in ophthalmic drug delivery is to bind hyaluronic acid to another polymer [192–194,196,208,209]. Hyaluronic acid is one of the most abundant glycosaminoglycans of the extracellular matrix. It has high water-binding capacity, viscous flow and pseudoplastic behavior. Hyaluronic acid is a mucoadhesive polymer able to prolong the residence time through two different mechanisms. First, like chitosan, hyaluronic acid forms hydrogen bonds with mucin. In addition, hyaluronic acid interacts with the CD44 receptors, which are widely expressed in human cornea and conjunctiva further contributing to the mucoadhesive effect [315]. Consequently, it is expected that the addition of this polymer to a carrier increases the bioavailability. Kalam encapsulated

Table 3
Pharmacokinetic parameters of indomethacin concentrations in cornea and aqueous humor after topical ocular instillation of the indomethacin-loaded carriers and the control solution. Adapted with permission of Elsevier from Calvo *et al.* [198].

Tissue/formulation	AUC (µg/min.per.g)	C_{max} (µper.g)	T_{max} (min)	$K_{el}10^{-3}$ (min^{-3})	$t_{1/2}$ (min)
Cornea					
Drug solution	292 ± 22	2.4 ± 0.2	30	8.2 ± 1.4	86 ± 13
Uncoated NPs	933 ± 177	10.9 ± 1.7	30	9.2 ± 1.4	77 ± 16
Coated NPs	1584 ± 254	18.9 ± 5.2	30	14.0 ± 1.6	49 ± 5
Aqueous humor					
Drug solution	17 ± 2	0.1 ± 0.1	30	8.7 ± 0.9	79 ± 8
Uncoated NPs	73 ± 21	0.7 ± 0.1	30	10.7 ± 1.8	65 ± 10
Coated NPs	114 ± 8	1.2 ± 0.1	30	12.8 ± 0.4	53 ± 1

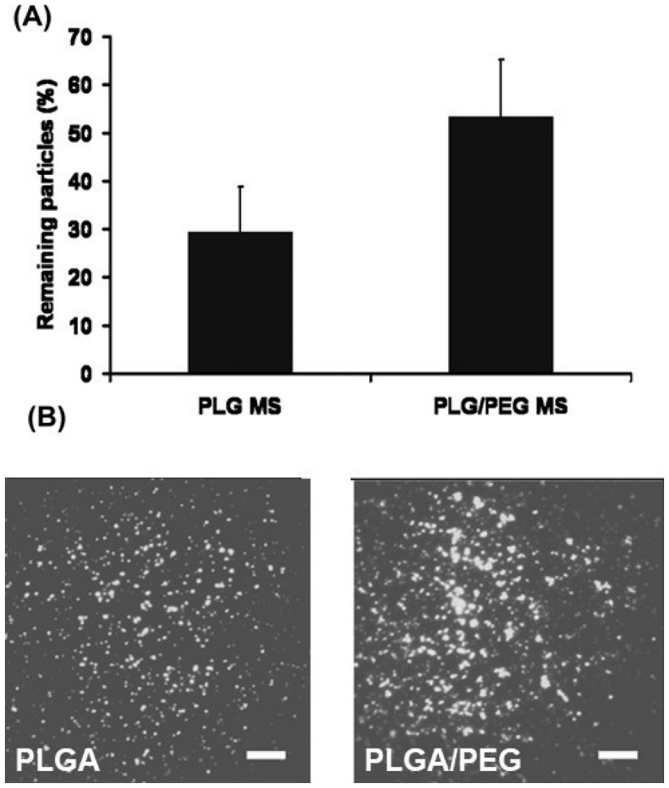

Fig. 14. *In vitro* mucoadhesion of microparticles. (A) Percentage of microparticles remaining on the mucous membrane. (B) Fluorescence images of poly (lactic-*co*-glycolic acid) (PLGA) and poly(lactic-*co*-glycolic acid)/poly(ethylene glcol) (PLGA/PEG) microparticles (MS) on the mucous membrane. Scale bars = 500 μm. Adapted with permission of Elsevier from Choy *et al.* [211].

dexamethasone in hyaluronan-coated chitosan NPs showing a prolonged precorneal retention of the nanosystem following its topical instillation in rabbits, as determined *in vivo* through the quantification of the drug in tear fluid [194]. Indeed, the $AUC_{0\text{-}240min}$ of chitosan- and hyaluronan-coated chitosan NPs were 1.43- and 1.67-fold higher than that of dexamethasone solution, respectively. This greater retention resulted in an increase in the bioavailability ($AUC_{0\text{-}24h}$) determined in aqueous humor of 1.83- and 2.14-times for chitosan- and hyaluronan-coated chitosan NPs with respect to a drug solution, respectively (Table 4), what reinforces the capacity of hyaluronic acid to prolong the residence time of the ODDS even more than chitosan alone. Importantly, these nanosystems were non-irritant according the Draize test [194]. Furthermore, hyaluronic acid contributed to the internalization of hyaluronic acid NPs by CD44 receptor-mediated endocytosis.

Table 4
Pharmacokinetic parameters of dexamethasone in aqueous humor after topical administration of a drug solution, drug-loaded chitosan nanoparticles and hyaluronic acid coated drug-loaded chitosan nanoparticles (mean ± SD, n = 3, for each time point). Reproduced with permission of Elsevier from Kalam [194].

Pharmacokinetic parameters	Values for		
	DEX solution	DEX-CS-NPs	HA-DEX-CS-NPs
$t_{1/2}$ (h)	2.032	3.679	4.529
T_{max} (h)	2.848	5.117	6.285
C_{max} ($ngmL^{-1}$)	329.704	354.473	356.712
$AUC_{0\text{-}24h}$ ($ngmL^{-1}.h$)	2548.563	4674.413	5451.366
$AUC_{0\text{-}inf}$ ($ngmL^{-1}.h$)	2553.891	4933.425	6099.187

This was clearly evidenced by De la Fuente *et al.* who performed internalization studies at 4 and 37°C [193]. Results showed an extensive internalization of hyaluronic acid/chitosan NPs and hyaluronic acid/chitosan oligomers NPs only at 37°C, indicating an energy-dependent endocytic pathway. To confirm whether CD44 is responsible for the receptor-mediated uptake, the receptor was blocked by the addition of the monoclonal antibody Hermes-1. After the receptor blockage, the uptake of the NPs was almost negligible, confirming the CD44 receptor-mediated internalization [193].

Chondroitin sulfate is another glycosaminoglycan of the extracellular matrix that can interact with the receptor CD44 and that can be used to form ocular carriers [37]. The addition of a hydrophilic polymer to a hydrophobic carrier can increase the encapsulation efficiency and reduce the burst effect of water-soluble drugs. However, the addition of hydrophilic polymers not always leads to this improvement [204]. It depends on the type and the molecular weight of the polymer used. For example, the encapsulation efficiency of BSA was 61-65% and 81-87% for PLGA and alginate-chitosan-PLGA NPs, respectively [229]. Moreover, the burst release of BSA at 1 h was 24% and 8% for conventional PLGA (50:50) and PLGA (70:30) NPs, respectively. Conversely, this value increased to 48% and 52% for the corresponding hybrid system [229]. Another form to capitalize on the combination of two different polymers is to perform a suitable blend in order to fine-tune the release profile. It is also possible to produce nanoparticle-in-microparticle delivery systems, a platform that is extensively reviewed elsewhere [316]. For example, Yandrapu *et al.* developed bevacizumab-coated PLA NPs encapsulated into porosifying PLGA MPs [213]. This system sustained the release of drug *in vitro* and *in vivo* for 4 months after intravitreal administration to rats [213].

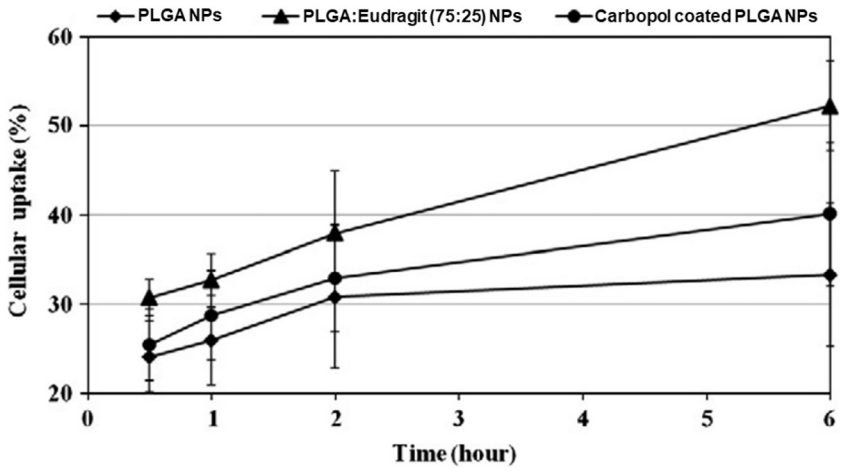

Fig. 15. Cellular uptake efficiency (%) of Nile red labelled nanoparticles. Reproduced with permission of Elsevier from Aksungur *et al.* [109].

Table 5
Pharmacokinetic parameters obtained after instilling different formulations containing pilocarpine.

Formulation	Peak time (min)	I_{max} (Δmm)	Duration (min)	AUC (Δmm min)	AUC_{rel}[a]	AUC_{rel}[b]
Drug	30	2.49 (0.46)	210	254.4 (53.3)	1.00	0.66
Drug-NPs	30	3.24 (0.64)	240	383.4 (81.5)	1.50	1.00
Drug in MC 0.50%	30	3.22 (0.56)	210	304.6 (64.4)	1.20	0.79
Drug in MC 1.00%	30	2.76 (0.72)	240	326.1 (121.4)	1.28	0.85
Drug in MC 1.50%	30	3.56 (0.51)	210	366.2 (62.1)	1.43	0.95
Drug-NPs in MC 0.50%	30	3.86 (0.86)	240	397.2 (93.3)	1.56	1.03
Drug-NPs in MC 1.00%	30	3.54 (0.86)	240	401.3 (148.4)	1.57	1.04
Drug-NPs in MC 1.50%	30	3.40 (0.47)	210	394.0 (88.0)	1.54	1.03
Drug in HPMC 0.40%	30	3.08 (0.90)	240	345.6 (146.4)	1.35	0.90
Drug in HPMC 0.60%	30	3.70 (0.53)	240	383.3 (54.8)	1.50	0.99
Drug in HPMC 1.10%	30	3.26 (0.37)	240	396.8 (61.2)	1.55	1.03
Drug-NPs in HPMC 0.40%	30	3.20 (0.14)	240	347.7 (55.4)	1.36	0.90
Drug-NPs in HPMC 0.60%	30	3.76 (0.74)	240	418.4 (129.2)	1.64	1.09
Drug-NPs in HPMC 1.10%	30	3.62 (0.73)	270	448.1 (105.6)	1.76	1.17
Drug in PVA 3.70%	30	2.87 (0.27)	210	271.5 (73.2)	1.06	0.71
Drug in PVA 4.85%	15	3.36 (0.49)	240	365.3 (102.3)	1.43	0.95
Drug-NPs in PVA 3.70%	30	3.12 (0.62)	240	329.0 (84.0)	1.29	0.85
Drug-NPs in PVA 4.85%	30	3.13 (0.63)	240	376.1 (105.8)	1.47	0.98
Drug in CMC 0.55%	15	3.26 (0.79)	240	371.2 (97.5)	1.45	0.96
Drug in CMC 0.90%	15	3.44 (0.68)	240	368.5 (101.5)	1.44	0.96
Drug in CMC 1.40%	15	3.52 (0.77)	240	383.5 (98.9)	1.50	1.00
Drug-NPs in CMC 0.55%	30	3.46 (0.50)	270	449.8 (27.8)	1.76	1.17
Drug-NPs in CMC 0.90%	30	3.22 (0.74)	270	404.9 (102.6)	1.59	1.05
Drug-NPs in CMC 1.40%	30	3.45 (0.21)	270	453.2 (89.0)	1.78	1.18
Drug in HA 0.30%	30	3.30 (0.67)	240	418.6 (101.8)	1.50	0.99
Drug in HA 0.80%	30	3.40 (1.20)	240	406.2 (144.9)	1.59	1.05
Drug in HA 1.00%	30	3.05 (0.82)	240	382.8 (155.6)	1.50	0.99
Drug-NPs in HA 0.30%	45	3.42 (0.59)	240	418.6 (101.8)	1.64	1.09
Drug-NPs in HA 0.80%	45	3.54 (0.69)	270	449.3 (150.4)	1.76	1.17
Drug-NPs in HA 1.00%	15	3.17 (0.09)	240	391.1 (17.9)	1.53	1.02
Drug in CP 0.15%	30	3.90 (0.19)	240	377.1 (62.9)	1.48	0.98
Drug in CP 0.50%	30	2.84 (0.65)	270	406.2 (110.6)	1.59	1.05
Drug in CP 1.00%	15	2.63 (0.29)	270	408.6 (75.0)	1.60	1.06
Drug in CP-NPs 0.15%	30	3.70 (0.53)	240	382.8 (48.9)	1.48	0.98
Drug in CP-NPs 0.50%	30	3.15 (0.60)	270	470.7 (87.0)	1.85	1.22
Drug in CP-NPs 1.00%	30	3.62 (0.54)	300	519.3 (76.8)	2.04	1.35
Drug in mucin 2.50%	30	3.68 (1.02)	210	380.4 (105.2)	1.49	0.90
Drug in mucin 4.50%	15	3.65 (0.34)	240	386.6 (26.6)	1.52	1.00
Drug-NPs in mucin 2.50%	15	4.26 (0.36)	300	631.8 (141.6)	2.48	1.64
Drug-NPs in mucin 4.50%	15	3.90 (0.45)	300	560.8 (143.5)	2.20	1.46

Peak time is the time at which the maximum value of miotic action is reached (I_{max}). Duration is duration of miosis. AUC_{rel}[a] is the relative bioavailability respect to the drug solution used as reference, AUC_{rel}[b] [0]is the relative bioavailability respect to drug loaded nanoparticles. Results are shown as the mean value (S.D.), (n = 5).

4. Ophthalmic vehicles to optimize the administration of ODDS

A key factor to achieve the best possible performance in ophthalmic delivery is the vehicle in which the polymer based-carriers will be administered. Their great versatility allows administration in multiple dosage forms like eye drop solutions, hydrogels or *in situ*-forming gels. Thus, it is possible to exploit the advantages provided not only by the polymeric carrier but also by the dosage form in which it is dispersed. For example, to increase the residence time of pilocarpine-loaded albumin NPs on the precorneal surface, Zimmer *et al.* dispersed them in liquid formulations containing viscous (methylcellulose, poly(vinyl alcohol) and hydroxypropylmethylcellulose) or bioadhesive polymers (hyaluronic acid, mucin, sodium carboxymethylcellulose and polyacrylic acid), as summarized in Table 5. However, this strategy did not substantially improve the bioavailability. Most of the nanoformulations showed similar AUC regardless of the vehicle used. Probably because the higher polymeric concentration, the higher the viscosity, inducing tearing. The best performances were obtained with bioadhesive polymers rather than with viscous ones [72]. Instead, an interesting approach to enhance the efficiency of polymer based-carriers is their dispersion in preformed hydrogels [38,53,118] or *in situ*-forming gels [47,106,108,195]. The former are hydrophilic polymers that swell in aqueous solutions. Although they prolonged the retention, its high viscosity produces blurred vision, crusting of eyelids and lachrymation that reduce patient compliance. Conversely, *in situ*-forming gels are instilled as eye drops and become a gel fast upon contact with the ocular surface in response to environmental changes such as pH, ionic strength or temperature. Among the different polymers available to produce this type of vehicles poloxamers, poly(*N*-isorpropyl acrylamide) (PNiPAAm) and poly(acrylic acid) stand out.

Poloxamers are nonionic poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblock copolymers widely used in pharmaceutical products. The PEO block is hydrophilic while the PPO is hydrophobic one. There are poloxamers with different molecular weights and PEO:PPO ratio that is identified in the name of each polymeric subtype. They are named as "P" followed by three digits: “P” comes from poloxamer, the first two digits multiplied by 100 give the approximate molecular weight of the PPO core and the last digit multiplied by 10 gives the percentage of PEO content. However, these biomaterials are also known by the BASF Corp. trade name Pluronic®. In this case, the code is a letter followed by two or three digits. The letter, ‘L’, ‘P’, and ‘F’ defines the physical form of the poloxamer: liquid, paste, or flakes, respectively. The first digit (two digits in a three-digit number) multiplied by 300 is the approximate molecular weight of the PPO core and the last digit multiplied by 10 is the percentage of PEO content [231,317]. Thus, it is possible to make the equivalences between these two ways of calling to this biomaterial (Table 6). Several of these copolymers have been approved as pharmaceutical excipients and in medical devices such as solutions for preservation of contact lenses.

Poloxamers were extensively used to prepare *in situ*-forming gels for

Table 6
Equivalences between poloxamer and Pluronic® regarding the composition.

Poloxamer	Pluronic®	≈PPO molecular weight (g/mol)	PEO content (%)
P 124	L44	1200	40
P 188	F68	1800	80
P 237	F87	2300	70
P 338	F108	3300	80
P 407	F127	4000	70

ocular delivery as they are transformed from a solution at room temperature to a semisolid gel at precorneal temperature (34.5°C) after dilution with tear fluid [74,76,166]. For example, Morsi *et al.* prepared ketorolac tromethamine-loaded Eudragit® RL100 NPs dispersed into a thermosensitive *in situ* gel based on Pluronic® F127 [148]. The gelation time and gelling temperature of the system was 37 ± 2.3 s and 33.0 ± 1.2°C, respectively. The concentration of poloxamer used to produce the vehicle was 20% w/v. Results showed that incorporation of NPs into the gel resulted in a significantly slower release rate of the drug and a significant improvement of ocular bioavailability (Table 7) [148].

PNiPAAm is another polymer that can be used to produce thermoresponsive hydrogels. Osswald *et al.* synthesized PNiPAAm-PEG-diacrylate hydrogels intended to contain PLGA MPs [102]. The vehicle was translucent below its transition temperature (33°C) and suspending the MPs within the vehicle did not alter its injectability through a 28G needle. Beyond the body temperature, the solution reverted to gel enabling the diffusion of the drug to the outside, while MPs remained within the gel. Therefore, the drug diffused first the MPs layer and then the barrier imposed by the gel, hence reducing not only the release rate but also the burst effect, along with prolonging the release from 154 to 196 days with respect to the drug-loaded MPs without the gel [102].

A remarkable disadvantage of PNiPAAm is that it has not been approved by the regulatory agencies for biomedical use. Other polymers used to produce *in situ* gels are those that respond to changes in pH. For example, chitosan and Carbopol® display sol-to-gel transition when the pH is raised above the pK_a. Hence, both gel at ocular pH [116,122,200]. For example, Gupta *et al.* developed levofloxacin-loaded PLGA NPs dispersed in an *in situ* gel prepared with chitosan [122]. Gamma scintigraphy was used to assess *in vivo* if the vehicle increases the retention of the drug in the precorneal surface. For this, levofloxacin was radiolabeled with Tc-99m. Results showed that the marketed eye drop solution cleared rapidly from the corneal area and reached the systemic circulation via nasolachrymal drainage system given that significant activity was detected in kidney and bladder 5 h after ocular administration. Conversely, free drug dispersed in the *in situ*-forming gel and drug-loaded NPs were retained at corneal surface for longer time. The best performance was obtained with an *in situ* gel containing drug-loaded NPs. Finally, there are polymers such as gellan gum and alginate that can be transformed into a gel in the presence of mono or/and divalent ions that are naturally present in the eye [49,145]. For example, *in situ* gelling dorzolamide-loaded chitosan NPs prepared with sodium alginate sustained more the release than simple counterparts and the free-drug dispersed in the *in situ* gel [49]. In fact, gamma scintgraphy showed that the formulation was well retained in the pre-corneal surface [49]. To sum up, it should be stressed that preformed gel as well as *in situ* forming gel, impacts not only the residence time of the drug in the absorption site which is required for non-mucoadhesive particles, but also the drug release kinetics. So, if encapsulating the drug in a particle is not enough to sustain the release, dispersing them in gels can be a suitable solution. Even, as was mentioned above these vehicles can also reduce or eliminate the burst effect.

To compare the ocular performance of these vehicles, Ibrahim *et al.* encapsulated celecoxib into NPs prepared with PCL, PLA and PLGA [77]. Each type of these NPs was dispersed in eye drops, *in situ*-gelling system and preformed gel. Results showed that the formulations that released the drug faster were eye drops, followed by gels, while *in situ*-gelling system displayed the lowest drug release rate. All the formulations were nontoxic [77]. However, despite *in situ* gels also sustained the *in vitro* release profile of brimonidine compared with preformed gels, these differences were not reflected in a significant difference of the *in vivo* hypotensive effect [40,53]. Therefore, it is worth noting that *in situ* forming gel is the most preferred over the preceding vehicles from a point of view of patient compliance, considering that a liquid dosage form is preferred over a semisolid one.

5. Regulatory aspects

The development of nano-drug delivery systems in general and for ocular delivery in particular advanced faster than the regulatory aspects. The advantages of the different nanoplatforms compared to conventional pharmaceutical formulations urgently require harmonization of production processes and quality control protocols, and legislation that ensures both safety and efficacy. Although several of the polymers above described are approved for ophthalmic administration by different regulatory agencies, when they are used to produce nanoparticles its biocompatibility can be altered. Thus, it is important to study cell toxicity of each nano-sized polymer-based carrier in the relevant cell types for the ocular route as a preamble to *in vivo* studies. Moreover, the toxicity of the drug can be increased due to the reduction of the particle size that increases drug absorption and alters biodistribution. Even though nowadays there is not a detailed legislation to regulate the use of nanomaterials in drug delivery, the US-FDA includes nanoplatforms under the term “complex product”, not being of biological origin [318]. If these complex products are novel products, they are evaluated through the new drug application (NDA) regulatory pathway which requires animal, clinical, and bioavailability studies. Conversely, if the complex products are generics or copies, they are authorized through the abbreviated new drug application (ANDA) regulatory pathway that only requires pharmaceutical equivalence and bioequivalence. However, sometimes these parameters are difficult to establish [318]. Currently, through a NDA, the US-FDA has given the permission to the company Aura Biosciences to begin clinical trials in patients with ocular melanoma using viral NP conjugates that bind selectively to cancer cells in the eye after its intravitreal injection [319]. Another clinical trial under way is based on the topical administrarion of urea-loaded NP eye drops to 51 patients with cataract which is sponsored by the Assiut University. In this case, NPs were prepared by the ionic gelation method between the tripeptide antioxidant

Table 7
Pharmacokinetic parameters of ketorolac tromethamine in aqueous humor of rabbits. Reproduced with permission of Elsevier from Morsi *et al.* [148].

Formula	C_{max} (ng/mL)	t_{max} (h)	$AUC_{(0-8)}$ (μg.h/mL)	$t_{1/2}$ (h)	Relative bioavailability (%)
Acular®	494.20 ± 17.63	1	1.097 ± 0.05	2.99 ± 0.21	100
E2	697.12 ± 22.51	2	2.228 ± 0.07	4.30 ± 0.53	203
NG2	553.32 ± 18.55	2	2.742 ± 0.11	11.57 ± 0.73	250

Acular® = commercial eye drops, E2 = ketorolac tromethamine loaded Eudragit® R100 nanoparticles, NG2 = in situ gel formulations containing ketorolac tromethamine loaded Eudragit® R100 nanoparticles.

glutathione and Pluronic® F127 [320]. Moreover, big pharma companies such as Glaxo Group LTD [321], Upjohn Co [322], Kala Pharmaceuticals [323], Novartis AG [324] and Merck [325], have filed patent applications involving polymeric NPs for ophthalmic administration. Moreover, there are already several granted patents in this field. This is the case of polymeric polyaspartamide-PEG micelles for use in the treatment of ophthalmic diseases by local administration of one or more drugs from antimicrobial, anti-inflammatory, antiglaucoma, antiviral, antiangiogenic and antioxidants agents. The patent belongs to the Italian company SIFI S.p.A [326]. Another example is an ophthalmic aqueous solution containing dexamethasone- loaded nanomicelles made with vitamin E succinate polyethylene glycol toxopherol (TPGS), patent of the Canadian company Aurinia Pharmaceuticals Inc [327]. This company has also protected the intellectual property of a topical aqueous solution of mixed micelles of vitamin E TPGS and octoxynol-40 encapsulating a calcineurin inhibitor or a mammalian target of rapamycin inhibitor [328]. The group of Maitra Amarnath of the University of Delhi has developed a sustained release and long-residing ophthalmic formulation comprising a polymeric micelle dispersion to administer ketorolac, indomethacin, nimesulide or a mixture of at least any two thereof [329]. Also, the Shandong Eye Institute from China patented eye drops containing cyclosporine A loaded-micelles formed with caprolactam polyethylene - polyvinyl acetate - PEG copolymer. This formulation not only reduces the irritation caused by the drug but also improves its corneal absorption, reduces dosing concentration, prolongs drug action time and reduces the frequency of administration, improving patient compliance [330]. The National Chiao Tung University protected a colloidal eyedrop gel containing carboxymethylhexanoyl chitosan (CHC) micelles. This formulation comprises a water- and a lipid-soluble drug inside and outside the micelle, respectively, being both for the treatment of glaucoma [331]. Moreover, the Korea Research Institute of Chemical Technology has protected a liquid formulation containing polymeric micelles of less than 10 nm, formed with triblock copolymers of PEO-PPO-PEO, encapsulating an immunosuppressant. This invention solves precipitation problems, does not blurr the vision and is stable for long storage without problems of toxicity and sterilization [332]. Also, there are granted patents related to polymeric NPs and MPs. For example, Shaker A. Mousa patented an ophthalmic formulation comprising NPs produced with PLGA, chitosan, chitosan-alginate, or NiPAAm-APMAH-AA, wherein APMAH is N-3-aminopropylmethacrylamide hydrochloride and AA is acrylic acid, for treating an ocular angiogenesis-mediated disorder. Each NP encapsulates sulfated non-anticoagulant heparin which is ionically or covalently bonded to the NP [333]. On the other hand, a patent related to polymeric MPs for controlled release was granted to the American company Lolab Inc. These MPs have a size from 50 μm to 2 mm and they are dispersed in an external matrix soluble in the lacrimal fluid [334]. The Spanish company Laboratorios CUSI SA patented an ophthalmic product containing nanocapsules of 100-5000 nm, characterized by a lipid core surrounded by a polymeric membrane of a polyacryl derivative or PCL. This invention is for topical or periocular administration of acyclovir, iododeoxyuridine, indomethacin, tetracycline, betaxolol or carteolol [335].

Other type of granted patents involves ocular formulations comprising nanocrystals (pure drug particles of nanometric size) immersed in a polymer solution that stabilize them. Thus, a patent was granted to one of the major companies of the ophthalmic market, Alcon (Novartis), for a topical aqueous ophthalmic nanosuspension of nepafenac in a formulation comprising a carbomer, guar and borate [336]. Remarkably, this formulation is already on the market; Ilevro™ for the treatment of pain and inflammation associated with cataract surgery (NDA regulatory pathway) [337,338]. Recently, Novartis received the assignment of another patent for a submicron suspension of a hydrophobic therapeutic agent that can be a tyrosine kinase inhibitor or a non-steroidal anti-inflammatory agent (nepafenac), stabilized in one or more cellulose polymers to be administered by intravitreal route [339]. Novartis is not the only company advancing the development of nanosuspensions. In fact, Allergan has received the grant of a patent for particles of a poorly soluble therapeutic agent, preferably triamcinolone acetonide, with a maximum dimension less than 3000 nm stabilized with hyaluronic acid or a salt thereof for treating an ophthalmic condition. This formulation is effective to form concentrated regions of the therapeutic agent in the retinal pigment of an eye of a human or animal epithelium when administered by intravitreal, subconjunctival, subtenon, retrobulbar or suprachoroidal injection [340]. The array of published and granted patents is encouraging since the protection of the intellectual property is usually the gateway to the market. Furthermore, it is worth noting that there are other types of polymeric drug delivery systems like hydrogels already commercially available. This is the case of the US-FDA-approved ReSure® Sealant from the company Ocular Therapeutix, Inc. This product gels *in situ*, protecting incisions in the immediate post-operative period when wounds are most vulnerable. The hydrogel gradually sloughs off in the tears during reepithelialization, so there is no need for removal [341].

6. Conclusions and perspectives

Advances in polymers science as well as in pharmaceutical technology enabled considerable progress in the optimization of ocular drug delivery field. Over the past 30 years novel DDSs were emerged demonstrating greater retention in the absorption site, sustained release over time, higher stability of the cargo and cellular uptake. These key factors enabled to surmount the obstacles imposed by the eye, leading to greater bioavailability. The benefits of polymers were potentiated even more by controlling the size of the ODDS and, in this framework, a broad spectrum of polymeric NPs and MPs were designed and evaluated in a variety of *in vitro* and preclinical models. The next challenge will be to realize their clinical potential for the benefit of patients. However, to accomplish this goal, scalable and cost-viable production processes that include sterilization are urgently required.

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