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In situ gelling systems: a strategy to improve the bioavailability of ophthalmic pharmaceutical formulations

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The low therapeutic efficacy exhibited by conventional ophthalmic solutions owing to precorneal elimination of the drug, drainage by gravity, nasolacrimal drainage, conjunctival absorption, and the absence of controlled release and of bioadhesive properties, can be overcome by the use of *in situ* gelling systems. The combination in the same formulation of different *in situ* gelling polymers with different stimuli-responsiveness mechanisms exploiting the unique physicochemical characteristics of the ocular tissues is one such strategy that has produced improved results compared with conventional systems. As we discuss here, the recent use of biodegradable and biocompatible polymers in colloidal carrier systems has proved to be the most effective strategy, resulting in the exponential increase of the bioavailability of the ophthalmic drugs.

The eye is unique in terms of its anatomical and physiological nature and defense mechanisms, which make the targeting of drugs to eye tissues one of the greatest challenges in drug delivery [1,2]. Topical instillation of drugs through eyedrops is the most important and well-accepted route of administration for the treatment of various eye disorders [1–3]. Conventional pharmaceutical formulations, such as solutions and suspensions, have many disadvantages, exemplified by rapid precorneal elimination, drainage by gravity, normal tear turnover, frequent instillation, enzymatic metabolism, nasolacrimal drainage, conjunctival absorption, and the absence of controlled release and of bioadhesive properties [3–7]. The residence time of most conventional ocular solutions is 5–25 min and only 1–10% of the topically applied drug is absorbed; in addition, a major part of the drug absorbed systemically results in systemic adverse effects [3,8,9].

The limited permeability of the ocular membranes contributes to the low absorption of ocular drugs, resulting in the short duration of the therapeutic effect and making a frequent dosing regimen necessary [3–5,10]. The instillation of highly concentrated eyedrops can cause adverse effects and cellular damage in ocular tissues [1].

One of the best strategies to increase or prolong the contact time of ophthalmic formulations with the ocular tissues is to increase the viscosity of the formulation using biodegradable and biocompatible polymeric hydrogels [1–4]. Hydrogels are 3D, hydrophilic, polymeric networks

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(homo- or copolymers) with physical properties that make them attractive for a variety of biomedical applications, particularly for the controlled local delivery of drugs [2]. The use of bioadhesive polymers result in an increase of ocular residence time, via their enhanced viscosity and mucoadhesive properties [2]. Given that the increase in the viscosity of ophthalmic formulations often causes blurred vision, it is essential to achieve the optimal range of viscosity as well as the most suitable rheological behavior that will ensure good efficacy and tolerance [3].

Other strategies to overcome the problems associated with the use of conventional eye drops is to use *in situ* gel-forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible phase transitions (sol–gel) and pseudoplastic behavior to minimize interference with blinking [11]. The *in situ* gel-forming polymeric formulations offer several advantages, such as sustained and prolonged action compared with conventional drug-delivery systems [5]. From a manufacturing point of view, the production of such devices is less complex and, thus, lowers the investment and manufacturing costs [5]. The phase transition of the *in situ* gelling systems on the eye surface can have different causes, including: temperature and the pH in the precorneal region or the electrolyte composition of the tear film [12,13]. Thus, over the past few years, researchers have successfully developed different formulations using ophthalmic drugs with different kinds of *in situ* gelling polymers (e.g. thermo, pH and electrolyte-responsive polymers), viscosity-increasing agents and isotonic agents [3].

Recently, different ophthalmic drug delivery strategies have been used to develop colloidal carrier systems with biodegradable polymers [1,4,8,14–17]. These include hydrogels, polymeric micelles, nanosuspensions and lipid-based nanocarriers (excluding emulsions, liposomes, cubosomes and niosomes) [8,14–16]. Nanocarriers, such as nanoparticles (NPs), have the capacity to deliver ocular drugs to specific target sites and, thus, could revolutionize the therapy of many eye diseases because they increase the contact time of the administered drug with its target tissue [15]. The use of drug-loaded NPs (DNPs), with dimensions between 1 nm and 0.5 μm, prepared with biodegradable polymers [e.g. chitosan (CS), aminated gelatin, dextran, collagen, hyaluronic acid, poly-L-arginine, 2-hydroxypropyl-β-cyclodextrin, methylated-β-cyclodextrin, polyacrylates, poly(lactide-co-glycolide) (PLGA), poly(lactide) (PLA), poly ε-caprolactone and albumin] that operate via different techniques (e.g. solvent evaporation, spontaneous emulsification/solvent diffusion, salting out/emulsification–diffusion, ionotropic gelation and desolvation, constitutes a versatile drug delivery system, with an ability to overcome physiological barriers and guide the drug to specific cells or intracellular compartments either by passive or ligand-mediated targeting mechanisms [14,15,17].

An important advance will be the development of NPs suspended in an *in situ* gelling vehicle that forms a gel at the external ocular surface [17]. Liposomes are highly biocompatible and biodegradable drug carriers that offer advantages such as prolonged drug retention and improved drug absorption [8,17,18]. However, despite these advantages, liposomes present some limitations, such as their surface charge, low drug loading, manufacturing difficulties for sterile products and the instability of the lipid aggregated on the mucin surface [8,17,18].

Thus, such strategies offer several potential advantages as delivery systems for ocular administration, such as improving the bioavailability of poorly soluble drugs, targeted and controlled release and the reduction of adverse effects [4,17].

Anatomy of the eye

The eye is a complex optical system that collects light from the surrounding environment, regulates its intensity through a diaphragm, focuses it through an adjustable assembly of lenses to form an image, converts this image into a set of electrical signals, and then transmits these signals to the brain through complex neural pathways that connect the eye via the optic nerve to the visual cortex and other areas of the brain [19]. This organ, illustrated in Fig. 1, comprises several different structures with specific physiological functions.

The wall of the eyeball (globe) comprises three primary layers: the sclera, or outer layer (the fibrous protective layer with the transparent cornea anteriorly), the uvea, or middle layer (having a vascular and nutritive function, and contains pigmented tissue comprising the choroid, ciliary body and iris) and the retina, or inner layer (which is the neural, sensory stratum of the eye) [3,20,21].

Corneal permeability is the most important factor in determining the drug concentration in aqueous humor [3,7]. The cornea is the clear surface of the outer eye, which is approximately 0.5 mm thick and comprises five layers: epithelium, Bowman's membrane, stroma, Descemet's membrane and the endothelium layer [7,22]. This structure has two main functions: it acts as (i) a barrier preventing germs, dirt and other harmful material from entering the inner eye and (ii) the outermost lens of the eye [7]. Despite the cornea comprising five layers, only three (the epithelium, stroma and endothelium) are significant with respect to barrier resistance [7,22]. The epithelium is a rate-limiting barrier for the transcorneal diffusion of most hydrophilic drugs [3,7]. The tight junctions of the corneal epithelium serve as a selective barrier for small molecules and prevent the diffusion of macromolecules via the paracellular route [3,22]. The stroma acts as diffusion barrier to highly lipophilic drugs owing to the hydrophilic nature of the former

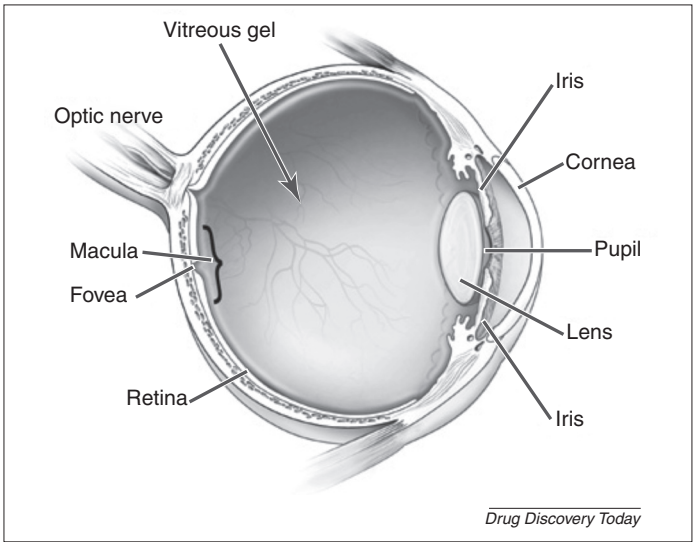

FIGURE 1

Anatomy of the eye globe. Courtesy: National Eye Institute, National Institutes of Health (NEI / NIH).

[3,7]. The endothelium is lipoidal in nature and does not act as a significant barrier to the transcorneal diffusion of most drugs [3,7].

The conjunctiva is a thin, vascularized mucus membrane that lines the inner surface of the eyelids and covers the anterior part of the sclera up to the cornea [3]. Conjunctival uptake of a topically applied drug from tear fluid is typically an order of magnitude greater than corneal uptake [3,22]. The mucin coat (approximately 2–3 μl mucus is secreted daily), secreted by the goblet cells of the conjunctiva, covers the conjunctiva and corneal surfaces of the eye, and hydrates, cleans, lubricates and serves as a defense against pathogens [3,16].

By contrast, the sclera is the outer supporting layer of the globe and extends from the limbus at the margin of the cornea anteriorly to the optic nerve posteriorly, where it is contiguous with the dural sheath of the optic nerve [3,20,23]. In fact, the composition of the sclera and cornea is identical, although one layer is clear and the other opaque [7,20–23]. The sclera acts as a protective layer, maintaining intraocular pressure and serving as the attachment site for the extraocular muscles [3,20,21].

Tear fluid composition

The eye has various protective mechanisms, including solution drainage, lacrimation, drug absorption via the vascularized conjunctiva, corneal barrier, melanin binding, aqueous humor flow and the blood–eye barrier [24,25]. The total volume of the lachrymal fluid is approximately 7–10 μl [14,16]. The corneal and the conjunctival surfaces are covered by continuous tear flow [25].

The tear film fulfills several important functions in the eye: (i) forming and maintaining a smooth refracting surface over the cornea; (2) maintaining a moist environment for the epithelial cells of the cornea and conjunctiva; (iii) having bactericidal properties; (iv) lubricating the eyelids; (v) transporting metabolic products (primarily oxygen and carbon dioxide) to and from the epithelial cells and cornea; (vi) providing a pathway for white blood cells in case of injury; and (vii) diluting and washing away noxious stimuli [26].

During each blink of the eye, water is mixed with oil and mucous components, and then distributed over the surface of the eye [26,27]. In fact, the eye surface is covered by a precorneal tear film composed of three layers: the superficial lipid layer, the middle aqueous layer and the deep mucous layer [26,27]. The oily or lipid portion (superficial lipid layer) is secreted by the Meibomian glands and the mucins, a family of glycoproteins that are produced by the conjunctival goblet cells [27]. This lipid layer comprises esters, triacylglycerols, free sterols, sterol esters and free fatty acids [26–28]. The function of the lipid layer is to lower the surface tension of the tear fluid and prevent evaporation [26,27]. The aqueous portion containing a salt solution is secreted by the main and accessory lacrimal glands [27]. This aqueous layer contains inorganic salts, glucose and urea, as well as biopolymers, proteins and glycoproteins [28]. The mucous layer, elaborated by the goblet cells of the conjunctiva, is the deepest stratum of the precorneal tear film and functions in coating the surface of the corneal epithelium, thus rendering it wettability by the aqueous tears, and in maintaining the stability of the film [28].

The presence of different electrolytes in the tear film can be used to promote the phase transition of *in situ* gelling systems [29,30]. Thus, the use of polymers sensitive to the presence of certain electrolytes in ophthalmic formulations promotes the increased ocular bioavailability of ophthalmic drugs by prolonging the contact time between the preparation and, therefore, the drug, and the corneal and/or conjunctival epithelium [29,30].

Physiological and pharmacokinetic limitations

The physiological limitations imposed by the protective mechanisms of the eye lead to low absorption of drugs and a consequent low therapeutic response exhibited by conventional ophthalmic solutions [31–33]. Although lacrimation and blinking are efficient protective mechanisms that keep the eye free of foreign substances, they prevent efficient ocular therapy [3,31–33]. Commercial eye-droppers deliver a drop volume of 25–56 μl (average 39 μl) [14,33]. When an eyedrop is instilled, the human conjunctival cul-de-sac (also known as the conjunctival fornix) might momentarily contain approximately 30 μl of volume, but the instilled solution is rapidly removed by spillage from the conjunctival sac or loss through the puncta to the lacrimal drainage system until the tears return to their normal volume (7–10 μl) [14,33]. This results in a tear turnover rate of 16% per minute during waking hours [16]. Upon instillation in the eye, the drug mixes with the fluid present in the tear film and has a short residence time of approximately 1–2 min in the film, because of the permanent production of lacrimal fluid (0.5–2.2 μl/min) [22]. Even though the lacrimal turnover rate is only approximately 1 μl/min, the excess volume of the instilled fluid flows to the nasolacrimal duct in a couple of minutes [1,16,34].

Another source of nonproductive drug removal is the systemic absorption of the drug instead of ocular absorption [34,35]. Systemic absorption can occur either directly from the conjunctival sac via local blood capillaries or after the solution has flowed into the nasal cavity [34]. The drug-containing tear fluid is carried from the lacrimal sac into the nasolacrimal duct, which empties into the nasal cavity, where the drug is then absorbed into the bloodstream [35]. This absorption leads to drug wastage and, more importantly, possible adverse effects [35].

In fact, only approximately 5% of the drug is absorbed into the cornea and the remaining either gets absorbed in the conjunctiva or flows through the upper and the lower canaliculi into the lacrimal sac [11,36]. The drug is then absorbed into the retina-choroid via an extracorneal, or scleroconjunctival route; the iris and ciliary body are supplied via both the transcorneal and the extracorneal pathways [3,22]. Drugs penetrate across the corneal epithelium via the transcellular or paracellular pathway [3,4].

Physicochemical drug properties, such as lipophilicity, solubility, molecular size and shape, charge and degree of ionization, affect the route and rate of permeation in the cornea [4]. The cornea is a heterogeneous barrier comprising the corneal epithelium (a major hydrophilic barrier), the stroma (a major lipophilic barrier) and the endothelium (a minor lipophilic barrier); therefore, lipophilic drugs can easily cross the corneal epithelium by a transcellular pathway (i.e. through the cells) either by facilitated transport or by diffusion through the lipid bilayer [37]. By contrast, it is difficult for hydrophilic drugs to pass from the tear film to the corneal and/or conjunctival epithelia and the paracellular entry through both corneal and noncorneal epithelia becomes the most important penetration route for hydrophilic drugs [37].

After eye drop administration, during the short contact of drug with the corneal surface, the drug partitions to the epithelium and, in the case of lipophilic compounds, remains in the epithelium and is slowly released to the corneal stroma and further to the anterior chamber [34]. The cornea releases the drug slowly into the aqueous humor, and then to the intraocular tissues (iris-ciliary body, lens, vitreous and choroid-retina) [3]. From the aqueous humor, the drug has easy access to the iris and ciliary body, where it can bind to melanin [33,34]. Melanin-bound drugs can form a reservoir that is released gradually to the surrounding cells, thereby prolonging drug activity [34].

Thereafter, the drugs are distributed from the aqueous humor to the intraocular tissues and eliminated mainly via aqueous-humor turnover through the chamber angle and Sclemm's canal and by the venous blood flow in the anterior uvea [4,34]. Ocular penetration via the scleroconjunctival route is more rapid (for a hydrophilic drug) than via the transcorneal route [38,39].

In summary, after eye drop administration, the drug can follow different pathways, including: (i) transcorneal permeation from the lacrimal fluid into the anterior chamber; (ii) noncorneal drug permeation across the conjunctiva and sclera into the anterior uvea; (iii) drug distribution from the blood stream via the blood–aqueous barrier into the anterior chamber; (iv) elimination of drug from the anterior chamber by aqueous-humor turnover to the trabecular meshwork and Sclemm's canal; (v) drug elimination from the aqueous humor into the systemic circulation across the blood–aqueous barrier; (vi) drug distribution from the blood into the posterior eye across the blood–retina barrier; (vii) intravitreal drug administration; (viii) drug elimination from the vitreous via posterior route across the blood–retina barrier; and (ix) drug elimination from the vitreous humor via an anterior route to the posterior chamber [34].

Strategies to improve the bioavailability of ophthalmic formulations

To overcome the drawbacks of conventional pharmaceutical formulations, it is necessary to develop formulations that not only prolong the contact time of the vehicle on the ocular surface, but also slow down the drug elimination [4,33].

Different strategies have been developed to increase the bioavailability of ophthalmic drugs by prolonging the contact time between the ophthalmic formulation and the ocular tissues. The following strategies have been used to improve the ocular bioavailability: (i) improvement of corneal permeability by chemical and pharmaceutical modification (e.g. prodrugs, penetration enhancers, ion pairs, iontophoresis and cyclodextrins); (ii) improvement of retention by vehicle (e.g. suspensions and ointments, viscous vehicles, bioadhesive vehicles and *in situ* gelling systems); (iii) improvement of retention by colloidal dispersion systems (e.g. liposomes, emulsions, NPs and nanocapsules); (iv) improvement of retention by solid polymeric matrix and devices (e.g. degradable matrices, nondegradable matrices, collagen shields and contact lenses, and membrane-controlled devices); and (v) implantable devices [4].

One of the most successful strategies is the increase in the viscosity of instilled solutions using polymeric gels [1,4,17,31,40]. Over the past decade or so, gels formed from natural, semisynthetic or synthetic polymers have been confirmed as vehicles for different types of pharmaceutical application [40].

However, the increase in viscosity can cause discomfort and blurred vision or foreign body sensation and, thus, it is important to assess the optimal range of viscosity as well as the most suitable rheological behavior to ensure good efficacy and tolerance of the formulation [41]. In fact, small increases in viscosity of an instilled ocular solution (i.e. less than 100 mPas) produce a modest retention of product for the first 20–50 s after instillation, after which viscosity has no role [41]. Based on published reports, formulations before gelling should have a viscosity of 5-1000 mPas and, after gelling in the eye, should have a viscosity of approximately 50–50,000 mPas [9]. A marked increase in the viscosity of formulations also results in rapid elimination of the instilled drug owing to an increase of reflex tears and reflex blinks [3,9,42,43]. The pseudoplastic character of precorneal tear film should be disturbed less by the administration of ophthalmic products [42]. The ocular shear rate is approximately $0.03\ s^{-1}$ during interblinking periods and $4250–28{,}500\ s^{-1}$ during blinking [42]. Thus, viscoelastic fluids with high viscosity under low shear rates and low viscosity under high shear rates, so-called 'pseudoplastic fluids', are often preferred [42,43].

Polymeric gels are classified into two distinct groups: bioadhesive or performed hydrogels and *in situ* gelling systems [31].

Bioadhesive hydrogels

The addition of specific polymers into ophthalmic pharmaceutical formulations causes an increase in the viscosity and mucoadhesive properties of the formulations and, therefore, an increase in the retention of drug in the ocular globe [32,33]. These kinds of polymers are capable of forming strong noncovalent bonds with the mucin coating biological membranes and remain in place as long as mucin is present [3,22]. Most of the bioadhesives developed for drug delivery systems comprise synthetic mucoadhesives, including water-soluble polymers that are linear chains, and water-insoluble polymers that are swellable networks joined by crosslinking agents [3,22]. An extended residence time of hydrogels would only be advantageous if part of the drug remained in the formulation and is slowly released during its stay in the cul-de-sac [3]. In fact, these kinds of polymer increase the drug residence time because the turnover of the mucus layer is slow (approximately 15–20 hours) [22].

The bioadhesive polymers most used in ophthalmic formulations are: polyvinyl alcohol (PVA), polyalcohol, polyacrylic acid (PAA), polycarbophil, polyvinyl pyrrolidone, hyarulonic acid, pullulan, chitosan (CS), polyethylene oxide (PEO), polymethacrylate, polyalkylcyanoacrylate systems, xanthan gum, carrageenan derivatives, cellulose derivates [methylcellulose, carboxymethylcellulose, hydroxypropylcellulose, hydroxyethylcellulose (HEC), and ethyl-HEC), polyacrylamide, poly(methylvinyl ether-maleic anhydride), pluronic acid and carboxyvinyl polymers [4,6,22,41]. For example, the addition of a polymer such as HEC has two benefits. First, the residence time on the corneal surface is significantly increased and, second, the rate of appearance in the nasolacrimal duct is slowed markedly [30].

In situ gelling systems

Interest in stimuli-responsive polymers is steadily gaining momentum, especially in the fields of controlled and self-regulated drug

delivery [4,17]. Stimuli-responsive or 'smart' polymers are macromolecules that display a significant physiochemical change in response to small changes in their environment [44,45]. The signs or stimuli that trigger the structural changes on smart polymers can be classified in three main groups: physical (temperature, ultrasound, light or mechanical stress), chemical (pH and ionic strength) and biological (enzymes and other biomolecules) [3,46,47]. These signs or stimuli can be artificially controlled (with a magnetic or electric field, light, ultrasound, etc.) or naturally promoted by the internal physiological environment through a feedback mechanism, leading to changes in the polymer net that enable drug delivery without any external intervention (e.g. pH changes in certain vital organs or related to a disease; temperature change or presence of enzymes or other antigens) or by the physiological condition [48–51].

The addition of smart polymers with drug molecules has advantages including the ability to administrate an efficient concentration of a certain drug on the right time and spot, a reduction in the adverse systemic reactions and an increase in the patient's adherence to the drug, thus enabling a reduction in the drug dose and, consequently, the costs [44].

Ophthalmic *in situ* gelling systems are viscous liquids that, upon exposure to physiological conditions, shift to a gel phase, causing an increase in the ocular residence time, via enhanced viscosity and mucoadhesive properties [42]. These systems are used to promote the increased ocular bioavailability of ophthalmic drugs by prolonging the contact time between the preparation and, therefore, the drug, and the corneal and/or conjunctival epithelium [42]. *In situ* gelling systems increase the viscosity by changing the pH or temperature owing to the presence of certain electrolytes in the tear film, and lead to an increase of drug bioavailability by slowing drainage [3]. Thus, ophthalmic formulations should include different kinds of *in situ* gelling polymer that exhibit reversible phase transitions and pseudoplastic behavior through the pH of the tears, the temperature at the eye surface, or the electrolytes present in the tear film [42]. In theory, one could take advantage of the intrinsic characteristics of the ocular globe to increase the retention time of the formulation and, therefore, cause an increase in the bioavailability of the drug used [3].

However, a crucial factor relating to this type of formulation containing *in situ* gelling systems is the time taken for the sol–gel transition. As a way of increasing the bioavailability of the formulation, the sol–gel transition of the formulation must be immediately after contact with the eye. To achieve this, three kinds of stimuli-responsive polymers can be used: thermoresponsive polymers, pH-responsive polymers and ion-activated polymers.

Thermoresponsive polymers

These smart polymers are sensitive to temperature and change their microstructural features in response to temperature change. These are the most studied, used and safe polymers in drug administration systems and biomaterials. The thermoresponsive polymers most widely used in ophthalmic formulations are: Poloxamers®, xyloglucan and poly(*N*-isopoprylacrilamide) (PNIPAAm).

Poloxamers® are non-ionic polymers (polyoxyethylene-polyoxypropylene-polyoxyethylene; PEO_n–PPO_n–PEO_n) and are temperature sensitive [28,45,52–54]. Block copolymers, based on PEO–polypropylene oxide (PPO) sequences [ABA-type triblock copolymers comprising PEO (A) and PPO units (B)], are a family of commercially available triblock copolymers that have the following trade names: Pluronics®, Poloxamers® or Tetronics® [28,55–58]. PPO forms a central hydrophobic core wherein methyl groups interact via van der Waals forces with substances undergoing solubilization [3,55–59]. However, water solubility is believed to result from hydrogen bonding interactions of ether oxygen with water molecules in the PEO block [3,55–59]. Owing to these interactions, Poloxamers are readily soluble in nonpolar organic solvents and, thus, established themselves in the formulation of dosage forms [3,59]. Aqueous solutions of Poloxamers in the presence of acids, alkalis and metal ions are very stable [3].

Pluronic® F-127 (PF-127) or Poloxamer 407 (P407) (copolymer $polyoxyethylene_{106}$–$polyoxypropylene_{70}$–$polyoxyethylene_{106}$) contains approximately 70% ethylene oxide, which contributes to its hydrophilicity [3]. PF-127 is a 12,000-Da copolymer with a PEO:PPO ratio of 2:1; it is non toxic, with low viscosity below 4 °C and forms a semisolid gel at body temperature [3]. The aqueous solution of PF-127 or P407 at a concentration of 20–30% (w/w) turns reversibly into gel at certain temperatures; that is, it becomes liquid at lower temperatures (4–5 °C) and turns into a gel at room temperature (this transformation is reversible, returning it to a liquid state at low temperatures) [3,28,60].

PF-127, a Poloxamer common in ophthalmic use, converts into a colorless, optical clarity and transparent gel at temperatures above 35 °C [3]. Showing mucomimetic properties as well as optical clarity, this Poloxamer can be successfully used as a tear substitute [3,22]. High concentrations of this polymer are required to form a stiff gel upon instillation in the eye [53]. Thus, ophthalmic formulations with a Poloxamer concentration >20% can cause eye irritation [3,22,53]. The strong concentration dependence of the sol–gel transition on temperature combined with the dilution that occurs in the eye means that the poloxamers cannot be used alone [3,22,53,61–64]. To reduce the total polymer content of a formulation and to improve the rheological behavior and gelling properties of the delivery system, a combination of various *in situ* gelling polymers with different phase transition mechanisms and viscosifying agents [e.g. methyl cellulose (MC), hydroxypropylmethylcellulose (HPMC) and sodium carboxymethylcellulose] have been used [3,22,53,61–64].

Many researchers have reported that the addition of isotonic agents, important in ophthalmic formulations (e.g. mannitol, sorbitol and sodium chloride) or viscosity-enhancing agents (e.g. HPMC, MC and carboxymethylcellulose sodium) promotes the increase in viscosity of PF-127 formulations and contributes to the decreased rate of drug release [61–64]. To choose a viscosifying agent, it is necessary to take into account the following aspects: viscosity, rheological behavior, mucoadhesive and wetting properties [61–64].

With this aim, El-Kamel [11] prepared PF-127 hydrogels with different concentrations (15, 20 and 25% w/w) formulated with timolol maleate (TM). The author then added different viscosity-enhancing agents (HPMC, MC and CMC Na) to the 15% w/w PF-127 hydrogel. The results showed that the slowest drug release was obtained from 15% PF-127 formulations containing 3% methylcellulose [11]. *In vivo* studies showed that the ocular bioavailability of TM, measured in albino rabbits, increased by 2.5- and 2.4 fold for 25% PF-127 gel formulation and 15% PF-127 containing 3% MC, respectively, compared with an 0.5% TM aqueous solution [11].

In another study, Wei *et al.* [65] developed a thermosetting gel with a suitable phase transition temperature with PF-127, PF-68 and sodium hyaluronan (mucoadhesive polysaccharide). Gamma scintigraphy demonstrated that the clearance of an optimized formulation containing 21% PF-127 and 10% PF-68 was significantly delayed with respect to a phosphate buffer solution [65]. A threefold increase in the corneal residence time was achieved in rabbits and the addition of sodium hyaluronate did not further improve the pre-corneal retention owing to a 50% decrease in gel strength [65].

With the same aim of increasing the permanence of an ophthalmic formulation in the ocular globe, Mayol *et al.* [66] tested the inclusion and influence of hyaluronic acid (HA) on the gelation properties of poloxamer blends. Mixtures of solutions of PF-127 (15% w/w), PF-68 (10–15% w/w) and HA (0.5–1% w/w) were formulated with acyclovir. This study demonstrated that the addition of low-molecular-weight HA into poloxamer blends is a useful tool for engineering thermosensitive and mucoadhesive polymeric platforms for sustained drug delivery [66]. On the basis of their viscoelastic properties, mucoadhesive forces, gelation behavior in simulated tear fluid and *in vitro* release properties (i.e. prolonged and controlled acyclovir release for more than six hours), poloxamer/HA gels are expected to be considered for a wide range of applications in ocular delivery [66].

The addition of cellulose derivates to PF-127 hydrogels promotes the increase of the bioavailability of the ophthalmic formulations [67,68]. Vodithala *et al.* [67] formulated PF-127 and MC with ketorolac tromethamine. PF-127 and MC at concentrations of 15% (w/w) and 2% (w/w), respectively, were found to be a better carrier system because it showed optimum gelation. With the increase in the concentration of PF-127 and MC, the gelation capacity increased and the sustained release of drug took more than five hours [67]. With the same aim, Darwhekar *et al.* [68] prepared different mixtures of solutions of PF-127 (15 and 20%, w/w) and HPMC (0.5, 1.0, 1.5% w/w) (Methocel K15M) formulated with dorzolamide hydrochloride and TM. The results showed that the formulation containing PF-127 (15% w/w) and HPMC (1.0% w/w) had better physiochemical characteristics, permeability properties and decreased frequency of administration with appreciable strength and safety.

To develop an ophthalmic drug delivery system for cyclosporine A (CsA) with reduced ocular irritancy and improved corneal penetration, Chen *et al.* [69] prepared glyceryl monoleate/poloxamer 407 liquid crystalline NPs. The results showed that CsA penetrated the cornea under the transportation of liquid crystalline NPs and that this kind of formulation exhibited excellent ocular tolerance in the ocular irritation test and increased corneal retention.

Different polysaccharides can be used as mucoadhesive ophthalmic vehicles, such as polygalacturonic acid, xyloglucan, xanthan gum, gellan gum, pullulan, guar gum, scleroglucan and carrageenan [1,4,5]. Xyloglucan gels have the potential for use for oral, intraperitoneal, ocular and rectal drug delivery [1,5]. Xyloglucan is a polysaccharide derived from tamarind seeds and comprises a (1-4)-β-D-glucan backbone chain, which has (1-6)-α-D-xylose branches that are partially substituted by (1-2)-β-D-galactoxylose [1,5]. When xyloglucan is partially degraded by β-galactosidase, the resultant product exhibits thermally reversible gelation by the lateral stacking of the rod-like chains [1,5]. The sol–gel transition temperature varies with the degree of galactose elimination [1,5]. It forms thermally reversible gels on warming to body temperature [5]. One of the most important characteristics of such polymers is the formation of macromolecular ionic complexes with drugs, which improved the bioavailability and lengthened the therapeutic effect compared with drug solutions [1].

Miyazaki *et al.* [70] investigated the xyloglucan and PF-127 sols as sustained drug release vehicles for pilocarpine hydrochloride administration in rabbits. *In vitro* release of pilocarpine from gels formed by warming xyloglucansols (1.0, 1.5 and 2.0%, w/w) to 34°C followed root-time kinetics over a period of six hours [70]. Sustained release of pilocarpine was observed with all gels and the duration of the miotic response increased with the increase in xyloglucan concentration [70]. The degree of enhancement of miotic response following the sustained release of pilocarpine from the 1.5% (w/w) xyloglucan gel was similar to that from a 25% (w/w) PF-127 gel [70].

Poly(*N*-isopoprylacrylamide) (PNIPAAm), from the poly(*N*-substituted acrylamide) family, is a synthetic temperature-sensitive polymer and the most studied and most used in drug delivery systems and biomaterials because it is soluble in water at room temperature [71]. Above the lower critical solution temperature (LCST–; 32°C) the solutions turns into a gel with a transition temperature similar to body temperature, with a predominance of hydrophobic interactions [71].

Hsiue *et al.* [72] investigated the use of PNIPAAm in controlled-release delivery systems for epinephrine for use in glaucoma therapy. When these formulations (linear PNIPAAm or a mixture of linear PNIPAAm and crosslinked PNIPAAm NPs containing epinephrine) were administrated to rabbits, there was a significant decrease in the intraocular pressure [72]. The decreased pressure response of the formulation based on linear PNIPAAm lasted six times longer than that of conventional eye drops, and eight times longer for the formulation based on the mixture of linear PNIPAAm and crosslinked NPs [72].

pH-responsive polymers

In the human body, there are remarkable changes in pH that can be used to direct therapeutic agents to a specific body area, tissue or cell compartment [73,74]. Owing to wide variations in the pH value of the physiological fluids, sol–gel transitions induced by pH changes seem to be an ideal approach for enhancing the pharmacological efficacies of the topical drug delivery, especially ophthalmic and intravaginal applications [73]. pH-sensitive polymers are polyelectrolytes that have in their structure acid (carboxylic or sulfonic) or basic groups (ammonium salts) that can accept or release protons in response to pH changes in the surrounding environment[74]. pH-responsive polymers most widely used in ophthalmic formulations are: PAA, polycarbophils, CS and cellulose acetate phthalate (CAP).

One of the most widely used polymers with thixotropic properties is PAA, whose aqueous solutions are less viscous and acidic in nature, and are transformed into gels upon increasing the pH [73,75]. pH-sensitive polymers named as polyacids or polyanions, such as PAA (Carbopol®) or poly(methacrylic) acid (PMAA) are polyanions that have in their structure many ionizable acid groups, such as carboxylic acid or sulfonic acid [76]. The carboxylic

groups accept protons at low pH values and release protons at high pH values [77]. Thus, when the pH increases, the polymer swells owing to the electrostatic repulsion of the negatively charged groups, delivering the drug molecules to the environment [45]. However, the pendent carboxylic acid groups at low pH values are not yet ionized and retain the drug in their interior; by contrast, drug delivery occurs as a result of the ionization of pendant groups of carboxylic acids, forcing the polymer to swell [45,77].

Thereby, a range of pH from 4.0 to 7.4 promotes an electrostatic, hydrophobic interaction and hydrogen bonding, leading to interdiffusion of the polymer [7]. The mucoadhesive properties of PAA result mainly from hydrogen bonding (between its –COOH groups and sialic acid –COOH groups of the mucin glycoprotein), whereas its hydrophobic interaction with mucin is not significant [1]. In fact, when anionic polymers interact with mucin, the maximum interactive adhesive force occurs at an acidic pH, suggesting that the mucoadhesive polymer in its protonated form is responsible for the mucoadhesion [7]. This promotes the interaction of the polymer with mucin, stabilizing a thick hydrogel structure [7]. The neutral pH value of the tears and the shielding of the carboxyl groups by cations present in the tear fluid diminish the interaction of the carbomer with the functional groups on mucin [7].

Rheological studies performed with various kinds of Carbopol® (974P NF, 980 NF and 1342 NF) demonstrated no significant differences in the interaction between these different carbomers and mucin, and concluded that this interaction depends on the mucin concentration in the ocular globe [1].

PAA gels in ophthalmic application demonstrated a retention time of approximately five hours and a long duration of action mainly because of the high yield stress that governs the shearing action of eyelids and movements of the eyeball *in vivo* [73]. The long retention time of the viscous gels is attributable to their high yield stress values, which enable them to withstand the *in vivo* shearing action of the eyelid and eyeball movements [73].

Schoenwald *et al.* [78] prepared gel formulations containing 2% pilocarpine hydrochloride with ethylene maleic anhydride, carbomer, HEC, polyacrylamide, ethylHEC, hydroxypropylcellulose, and poly(methylvinyl ether-maleic anhydride). At carbomer concentrations above 3%, miosis durations increased 1.5-fold [78]. It was reasoned that the increased duration was a consequence of the increased yield value of the gel, such that appreciable *in vivo* thinning of the gel does not take place with eyelid and/or eyeball movements [78]. As a result, the residence time of the drug in the eye would be expected to increase, thus promoting an increased duration.

In ophthalmic formulations with high concentrations of PAA, the low pH of the PAA solution could cause damage to the surface of eye before being neutralized by the lacrimal fluid [6]. This problem was solved by partially combining PAA with HPMC or other inert polymers (viscous-enhancing polymers), without compromising the overall rheological properties of the delivery system, which resulted in pH-responsive polymer mixtures that were solutions at pH 4 and gels at pH 7.4, that is, this formulation resulted in low-viscosity liquids at pH 4.0 and transformed into stiff gels with plastic rheological behavior and comparable viscosities when the pH increased to 7.4 [79]. HPMC is used in combination with Carbopol® to impart viscosity to the Carbopol® solution, while reducing its acidity [79]. The HPMC–PAA combination demonstrates properties suitable for formulation as liquid ophthalmic delivery systems, which upon instillation into the cul-de-sac of the eye can undergo *in situ* phase transition to form gels capable of sustained TM release [79].

Srividya *et al.* [80] prepared mixtures of solutions with PAA (Carbopol®940) (0.2–0.5%, w/w) and hydroxypropylmethylcellulose (Methocel E50LV) (1.0–1.5%, w/w) with ofloxacin (an antibacterial agent). The developed formulation was therapeutically efficacious, stable, nonirritant and provided sustained release of the drug over an eight-hour period [80]. Kumar *et al.* [81] prepared different ophthalmic formulations with Carbopol® and methylcellulose. An increase in pH from 4.0 to 7.4, or temperature from 25°C to 37°C, resulted in an increase in viscosity [81]. Among the compositions studied, a solution containing 1.5% methylcellulose and 0.3% Carbopol® was found to form a strong gel under simulated physiological conditions [81]. A possible mechanism of the thermal effect could be a decrease in the degree of hydration of methylcellulose, concomitantly with a conformational change of the polymer structure with the increase in temperature [22].

In another study, Wu *et al.* [82] formulated an *in situ* pH-triggered gelling system prepared with Carbopol®974P (0.3% w/w) and hydroxypropylmethylcellulose E4M (0.6% w/w) carrying the drug baicalin (with anti-inflammatory and anticataract effects on eye tissues). The rheological behavior showed a significant enhancement in gel strength under physiological conditions, and the formulation provided sustained release of the drug over an eight-hour period [82]. The results also demonstrated that an *in situ* pH-triggered gelling system was better able to keep baicalin stable and to retain drug release to enhance the ocular bioavailability than were marketed baicalin eye drops [82].

The potential of liposomes and Carbopol® as ophthalmic drug delivery systems was investigated by Durrani *et al.* [83], who studied the effect of Carbopol® 1342 on the *in vivo* ocular bioavailability of pilocarpine nitrate entrapped in liposomes. The results showed increased ocular bioavailability based on the intensity and duration of miotic response compared with uncoated vesicles [83]. This study demonstrated that prolonged retention of liposomes and the entrapped substance at the pre-corneal site can enhance drug delivery to the eye [83].

By contrast, Oechsner and Keipert [84] formulated the polymer PAA (0.1–0.2%, w/w) with another polymer, polyvinylpyrrolidone (PVP), at a concentration of 6% (w/w). The addition of PVP caused a significant decrease in the apparent viscosity of the combination [84]. This is important for decreasing the ocular irritation caused by the high viscous gel systems prepared only with PAA [84]. The study also showed that the formulation had a low viscosity and a high mucoadhesion index compared with the monopolymer PAA [84] preparation, leading to the conclusion that the combination of the two polymers, PAA and PVP, could be advantageous for the treatment of dry eyes [84].

Polycarbophil is the lightly crosslinked commercial form of PAA and exhibits stronger mucoadhesion, similar to that of Carbopol® [7]. This kind of polymer is used to prolong the pre-corneal residence time owing to *in situ* gel formation and mucoadhesion and is safe to use in topical ophthalmic pharmaceutical products (tested in albino rabbits at concentrations of 0.6–1.3%), is indicated for chronic use and for the treatment of conditions with compromise of the ocular surface [85]. In fact, polycarbophil is an anionic bioadhesive that is water insoluble, but its high swelling

capacity in a neutral medium enables the entanglement of the polymer chains with the mucus layer [7,85]. The resulting polymer swells and incorporates large quantities of water at neutral pH [22]. By contrast, the nonionized carboxylic acid groups of polycarbophil bind to the mucin by means of hydrogen bonds [1,7].

Gentamicin was investigated by Lehr *et al.* [86] using polycarbophil to improve the ocular delivery of topically applied hydrophilic drug in rabbits. The results showed that the polymeric formulation increased the uptake of gentamicin by the bulbar conjunctiva twice, compared with an aqueous control formulation [86].

The potential of liposomes as ophthalmic drug delivery systems was investigated by Nagarsenker *et al.* [87] who compared the pupil dilatory effect of tropicamide by topical instillation, in the rabbit eye, in the form of a solution and various drug-loaded liposomal forms [i.e. neutral liposomes, positively charged liposomes and neutral liposomes dispersed in 0.25% (w/w) of polycarbophil gel]. The results showed that the positively charged liposomal formulation and liposomes dispersed in polycarbophil gel were more effective than neutral liposomal dispersion [87].

To investigate the permeation-enhancing effect of thiolated polycarbophil on the cornea of rabbits *in vitro*, Hornof and Bernkop-Schnürch [88] compared the transcorneal permeation of sodium fluorescein and dexamethasone phosphate with thiolated polycarbophil and with unmodified polycarbophil. The modification of polycarbophil was achieved via covalent attachment of L-cysteine mediated by a carbodiimide [88]. Polycarbophil–cysteine showed a 2.2-fold and 2.4-fold increase in the transcorneal permeation of sodium fluorescein and dexamethasone phosphate, respectively, compared with the unmodified polycarbophil [88].

Cationic polymers have good mucoadhesive properties because of their ability to develop molecular attraction forces by electrostatic interactions with the negative charges of the mucus [1,5]. For this reason, CS was chosen to prepare ophthalmic *in situ* gelling systems with excellent results.

CS is a polysaccharide comprising copolymers of glucosamine and *N*-acetylglucosamine [1,4,5]. It is a biodegradable, thermosensitive, polycationic polymer obtained by alkaline deacetylation of chitin, a natural component of shrimp and crab shell [5]. This polymer has several advantages, among them, excellent tolerance after topical application, bioadhesive properties, hydrophilicity, good spreading over the entire cornea, antimicrobial and wound-healing properties, and pseudoplastic and viscoelastic behavior (desirable for ocular drug administration) [1,37,89].

CS has the ability to convert into hydrogel at an ocular pH (pH 7.4) and to develop secondary chemical bonds, such as hydrogen bonds or ionic interactions between the positively charged amino groups of CS and the negatively charged sialic acid residues of mucins, depending on environmental pH [1,89]. The mucoadhesive performance of CS is significantly higher at neutral or slightly alkaline pH, as found in the tear film [1]. In fact, at acidic pH, the amino groups are protonated, which promotes solubility, whereas CS is insoluble at alkaline and neutral pH [1].

Many researchers attribute other characteristics to CS. These include the fact that it acts as a penetration enhancer that increases the transcorneal permeation of the drug by opening the tight junctions between epithelial cells or by intracellular routes or through a mechanism based on the positive charges of the polymer, which interact with the cell membrane, resulting in a structural reorganization of tight junction-associated proteins [90].

To prolong the release of acyclovir and to increase its ocular bioavailability, Genta *et al.* [91] prepared drug-loaded CS microspheres by an emulsification technique. Results showed that 90% of the particles were approximately 25 μm in size and the *in vitro* dissolution profile of the drug was slow. *In vivo* ocular studies on rabbits showed a prolonged high concentration of acyclovir. Thus, this microparticulate drug carrier shows promising effects in the topical administration of acyclovir to the eye [91].

Calvo *et al.* [92] combined the features of poly-ε-caprolactone nanocapsules as ocular carriers with the advantages of the cationic mucoadhesive CS and poly-L-lysine (PLL) coating. The results showed that, even though PLL and CS displayed a similar positive surface charge, only CS-coated nanocapsules enhanced the ocular penetration of indomethacin with respect to uncoated nanocapsules [92].

In another study, Felt *et al.* [93] prepared different formulations with CS (0.5–1.5%, w/w) and tobramycin (0.3%, w/w). The results showed that the addition of CS promoted a threefold increase in the pre-corneal residence time of tobramycin compared with the commercial solution of the drug [93].

Majumdar *et al.* [90] demonstrated that CS with a concentration of 0.2 and 0.1% (w/w) increased corneal permeability across isolated rabbit cornea by 5.8- and 3.1-fold, respectively, whereas, at 0.02% (w/w), CS did not exhibit a statistically significant effect. Cationic polymers are likely to be superior mucoadhesives because of an ability to develop molecular attraction forces by electrostatic interactions with the negative charges of the mucus [1].

Over recent years, studies of novel ocular drug delivery systems have been reported, such as *in situ* gels, microemulsions, microspheres, liposomes and solid lipid NPs (SLN), all of which aim to prolong the pre-ocular retention and promote the absorption of the drug [1,4,8,14–17]. CS micro- or NPs have a higher pre-corneal retention compared with CS solutions and, depending on the size, the NPs can enter the corneal epithelium to a certain depth by a paracellular or transcellular pathway [1]. De Campos *et al.* [94] prepared CSNPs as a new vehicle for the improvement of the delivery of drugs to the ocular mucosa. These NPs were formulated with CyA. *In vitro* release studies revealed a fast release during the first hour followed by a more gradual drug release during a 24-hour period [94]. *In vivo* experiments showed that, following topical instillation of CyA-loaded CS NPs to rabbits, it was possible to achieve therapeutic concentrations in external ocular tissues (i.e. cornea and conjunctiva) for at least 48 hours while maintaining negligible or undetectable CyA levels in inner ocular structures (i.e. iris/ciliary body and aqueous humor), blood and plasma [94].

Diebold *et al.* [95] prepared a colloidal nanosystem with the potential to deliver drugs to the ocular surface. This nanosystem, liposome–CSNP complex (LCS-NP), was created as a complex between liposomes and CSNPs (CS-NP) [95]. The conjunctival epithelial cell line IOBA-NHC was exposed to several concentrations of different LCS-NP complexes to determine their cytotoxicity [95]. The *in vitro* toxicity of LCS-NP in the IOBA-NHC cells was low. LCS-NPs were identified inside IOBA-NHC cells after 15 min and inside primary cultures of conjunctival epithelial cells after 30 min [95]. Fluorescence microscopy of the conjunctiva revealed

strong cellular uptake of LCS-NP *in vivo* and less intensive uptake by the corneal epithelium [95].

Polylactic acid (PLA) hydrophobic NPs have been successfully coated with CS through the use of an amphiphilic derivative of CS [cholesterol-modified CS (CS–CH)] for the delivery of the hydrophobic immunosuppressive agent rapamycin (RAPA) [96]. The results showed the excellent immunosuppressive effect of the RAPA-loaded NPs compared with the eye drops, as noted by the significant increase in the median allograft survival time of rabbits bearing corneal allografts [96].

With the same aim, Li *et al.* [97] developed liposomes coated with low-molecular-weight CS. The CS coating changed the liposome surface charge and resulted in an improved physicochemical stability at 25°C over a 30-day storage period [97]. The ocular bioadhesion property was evaluated by rabbit *in vivo* pre-corneal retention, and CS-coated liposomes achieved a significantly prolonged retention compared with noncoated liposome or drug solution [97]. The CS coating also displayed a potential penetration-enhancing effect for transcorneal delivery of the drug [97].

Luo *et al.* [98] developed CS oligosaccharides (COS)-coated nanostructured lipid carriers (NLC) for the ocular drug delivery of flurbiprofen. For COS-coated NLCs, appropriate amounts of the polymer was dissolved in water to form a series of concentrations (0.1%, 0.3%, 0.5% and 1%, w/w), then mixed with flurbiprofen-loaded NLC dispersions. This kind of formulation promoted an improved penetration rate to the corneal tissues and had superior mucoadhesive properties, which were beneficial to the ocular drug delivery system [98].

Cellulose acetate phthalate (CAP) latex is a low-viscosity aqueous dispersion that undergoes spontaneous coagulation and/or gelation in the conjunctival cul-de-sac, because of an increase of the local pH [22]. It is a polymer with potentially useful properties for sustained drug delivery to the eye, because latex is a free-running solution at a pH of 4.4 that undergoes coagulation when the pH is increased by the tear fluid to pH 7.4 [22]. The pH change of 2.8 units after instillation of the native formulation (pH 4.4) into the tear film leads to an almost instantaneous transformation of the highly fluid latex into a viscous gel [7]. This massive swelling of the particles results from the neutralization of the acid groups contained in the polymer chain [31].

Although CAP latex does not induce visible irritation in rabbit eyes, other researchers have tested the eye irritation of several formulations [1,31]. The results showed the importance of the pK_a of the pseudolatex and of the pH conditions [31]. The high CAP concentration (30%) and the low pH of the preparation can elicit discomfort in some patients [1].

Gurny [99] prepared cellulose acetate hydrogen phthalate NPs with pilocarpine. The pH of the formulation was 4.5 but after instillation into the eye, the tear fluids rapidly shifted the pH of the applied liquid to 7.2, causing dissolution of the polymeric particles and an increase in the viscosity of the formulation that prevented a rapid washout of the formulation and the action of pilocarpine was considerably prolonged [99]. In another study by the same author, the addition of 0.125% HA to the previous formulation showed a more pronounced decrease of drug elimination of pilocarpine, which was also confirmed by elimination kinetics [100].

The association with poloxamer has also been evaluated and it has been noted that CAP latex is well tolerated when used alone, but becomes irritating when mixed with poloxamer [31].

Ion-activated polymers

These kinds of polymer can undergo phase transition in the presence of different electrolytes. The tear fluid presents, in its constitution, different electrolytes that promote the sol–gel transition of certain polymers, increasing with this the drug residence time and the bioavailability in the ocular globe. Ion-activated polymers most widely used in ophthalmic formulations are gellan gum and sodium alginates (ALG).

Gellan gum is an anionic polysaccharide (an anionic deacetylated exocellular polysaccharide secreted by *Pseudomonas elodea* with a tetrasaccharide-repeating unit of one α-L-rhamnose, one β-D-glucuronic acid and two β-D-glucuronic acid residues) formulated in aqueous solution, which undergoes a sol–gel transition under the influence of an increase in ionic strength [5]. The gelation of this polymer increases proportionally to the amount of either monovalent or divalent cations present in the lacrimal fluid [5,31]. As a consequence, the usually reflex tearing, leading to a dilution of viscous solutions, further enhances the viscosity of the formulation by increasing the tear volume and, thus, the cation concentration [31].

The electrolytes of the tear fluid, and especially Na^+, Ca^{2+} and Mg^{2+} cations, are particularly suited to initiate gelation of the polymer when instilled as a liquid solution into the cul-de-sac [12]. The interactions of the cations presents in the tear fluid and the negatively charged polysaccharide promotes a crosslinking structure that improves the residence time and the bioavailability of the formulation in the ocular globe [12]. In this particular case, the improved formulation incorporated gellan gum, which undergoes a thermal and cation-triggered phase change from liquid to gel [12,30]. This material forms a depot on the sclera and is retained for significant lengths of time [30]. Paulsson *et al.* [101] demonstrated in their studies that Na^+ was the most important gel-promoting ion *in vivo*. In detail, the gelation of this polymer involves the formation of double helical junction zones followed by aggregation of the double helical segments to form a 3D network by complexation with cations and hydrogen bonding with water [5].

In vitro studies in rabbits have shown that solutions of gellan gum are well tolerated by the eye [102]. Nevertheless, more recent human studies frequently report, in some cases, blurred vision owing to the high viscosity of the gel and possibly because of the formation of an opaque matrix induced by gellan–cation interaction in the buffer [31]. One thing to keep in mind is that the cation concentration in tears is lower in rabbits, which, along with their lower basal tear turnover, can result in a markedly different gelation of, for example, gellan gum, from that found in humans [31,102].

It is widely accepted that non-Newtonian vehicles such as gellan gum, HA and carbomers are more effective than Newtonian formulations containing PVA or celluloses in a similar viscosity range [31].

It was found that gels are formed in tear fluid even when the concentration of Gelrite®, the trademark of gellan gum, is only 0.1% (w/w) and samples with concentrations of Gelrite® of 0.5–1% (w/w) do not require more than 10–25% of the ions in tear fluid

TABLE 1

Examples of the use of two or more stimuli-responsive polymers in ophthalmic pharmaceutical systems

Pharmaceutical formulations	Results	Refs
Mixtures of solutions of Poloxamer 407 (21%, w/w), Poloxamer 108 (5%, w/w) and Carbopol® 1342P NF (0.1% or 0.2%, w/w) formulated with puerarin	The incorporation of Carbopol® 1342P NF not only did not affect the pseudoplastic behavior with hysteresis of the poloxamer analogs solution, leading to a higher shear stress at each shear rate, but also enhanced the mucoadhesive force significantly *In vitro* release studies demonstrated diffusion-controlled release of puerarin from the combined solutions over a period of eight hours. *In vivo* evaluation indicated that combined solutions had better ability to retain drug than poloxamer analogs or Carbopol® alone	[106]
Mixtures of solutions of PF-127 (10–25%, w/w) and CS (0.1–0.3%, w/w) formulated with ciprofloxacin	The formulation comprising 15% Pluronic and 0.1% low-molecular-weight chitosan, with the highest release efficiency (46.61 ± 0.41%) and an acceptable mean release time (1.94 ± 0.27 hours), is suggested as a suitable ophthalmic preparation for sustained release of ciprofloxacin	[107]
Different mucoadhesive CS–ALG nanoparticulate formulations of gatifloxacin were prepared using the following composition: CS (0.10–0.30% w/w), ALG (0.20–0.60% w/w), gatifloxacin (0.01–0.10%, w/w) and Pluronic® F127 (0.50% w/w)	*In vitro* release studies showed that the drug is released from the optimized formulation over a period of 24 hours in a sustained release manner, primarily by nonFickian diffusion This formulation is a viable alternative to conventional eye drops because of its ability to sustain release of the drug	[36]
Formulation of Carbopol® 940 (0.4%, w/w) and CS (0.5%, w/w) with TM	This in situ gelling system was in liquid state at room temperature at pH 6.0 and underwent rapid transition into the viscous gel phase at the pH of the tear fluid (pH 7.4) This kind of formulation prolonged the residence time of TM in the cul-de-sac and increased its bioavailability compared with a commercial TM solution. It also decreased the drug systemic absorption	[108]
Mixtures of solutions of Poloxamer 407 (14–20%, w/w) and CS (0.5–1.5%, w/w)	The results showed that CS improves the mechanical strength and texture properties of poloxamer formulations and also confers mucoadhesive properties in a concentration-dependent manner After a 10-min instillation of the poloxamer:CS16:1 formulation in human eyes, 50–60% of the gel was still in contact with the cornea surface, which represents a fourfold increased retention compared with a conventional solution	[109]
Pluronic® F-68 (PF-68) (14–16%, w/w) and Gelrite® (0.3–0.7%, w/w) as independent variables used in combination with PF-127 (15%, w/w), containing moxifloxacin hydrochloride as a model drug	PF-68 loading with PF-127 had a significant effect on gelation temperature of the formulation and to be important for gel formation at temperatures of 33–36°C Gelrite® loading had a positive effect on bioadhesion force and gel strength and was also found to help control the release rate of the drug	[62]
Nanosuspension of forskolin nanocrystals (10%, w/w) in Noveon® AA-1 polycarbophil (1.0%, w/w)/poloxamer 407 platforms (30%, w/w)	*In vitro* release experiments indicated that the optimized platform was able to prolong and control forskolin release for more than five hours *In vivo* studies on dexamethasone-induced glaucomatous rabbits indicated that the intraocular pressure lowering efficacy for nanosuspension/hydrogel systems was 31% and lasted for 12 hours, significantly more than the effect of traditional eye suspension (18%, 4–6 hours)	[110]
Evaluation of the interaction of the PLGA–CS nanoplexes formulated with fluorescent rhodamine	Data from *ex vivo* and *in vivo* studies showed that the amounts of rhodamine in the cornea were significantly higher for nanoplexes than for a control rhodamine solution, being fairly constant for up to 24 hours PLGA–CS nanoplexes interacted and remained associated with the ocular mucosa for an extended period of time and, thus, are promising carriers for enhancing and controlling the release of drugs to the ocular surface	[111]
Formulation and evaluation of CS solutions (0.5, 1.0 or 1.5%, w/w) and *in situ* forming gels comprising poloxamer 407 (16%, w/w) and CS (0.5, 1.0 or 1.5%, w/w) formulated with fluconazole	*Ex vivo* permeation studies across porcine cornea demonstrated that the formulations have a permeation-enhancing effect that is independent of CS concentration in the range 0.5–1.5% (w/w) The CS solutions alone showed the greatest *ex vivo* drug permeation; however, the poloxamer–CS formulation presented similar *in vivo* performance to the CS solution at 1.0%; both formulations showed sustained release and an approximately 3.5-fold greater total amount of fluconazole permeated when compared with simple aqueous solutions of the drug	[112]
Preparation and evaluation of O/W NEs for ophthalmic administration of TM. The O/W NEs were prepared with different polymers, such as: PVA 14000 and 85000, Pluronic® F68, low and medium-viscosity CS, PEG and HEC	The use of thickening and/or mucoadhesive polymers (CS, HEC, PVA, and PEG) significantly increased the viscosity of the preparation; therefore, this is likely to be able to achieve *in vivo* prolonged residence time on the preocular surface The introduction of CS (a positive charged mucoadhesive polymer) into emulsions resulted in increasing TM permeation, probably because of the interaction of CS with corneal epithelial cells	[113]
Preparation of tropicamide-loaded tamarind seed xyloglucan (TSX) nanoaggregates optimized the use of a face-centered central composite experimental design, using the concentrations of TSX and Poloxamer-407 as independent variables	The optimal concentrations of TSX and poloxamer were 0.45% (w/w) and 0.5% (w/w), respectively The optimized formulation of tropicamide-loaded TSX nanoaggregates showed a significantly higher corneal permeation of tropicamide across the isolated goat cornea compared with commercial conventional aqueous formulations	[114]

to form gels [101]. A correlation between the rheological and the mucoadhesive properties of different Gelrite® gels was demonstrated by Carlfors *et al.* [103]. Solutions of Gelrite® at different concentrations (0.4–1.2%, w/w) containing 0.00–0.02% (w/w) benzalkonium chloride and various amounts of glycerol (0–4% w/w) were prepared by heating the dispersions to 90 °C for 20 min while stirring [103]. The addition of glycerol and benzalkonium chloride aimed to achieve solvents with different osmolalities [103]. The elastic moduli of the gels increased with increasing concentration of electrolytes, but at a physiological concentration of the electrolytes, the elasticity of the gels was independent of Gelrite® concentration [103]. The human contact times increased up to 20 hours with decreasing osmolality of the formulations [103].

To develop a formulation that increased the pre-corneal residence time, Dickstein *et al.* [104] developed a comparative study to evaluate the effect of a placebo, a 0.5% (w/w) aqueous timolol (timolol solution) and a 0.5% (w/w) timolol suspension that forms a gel on application to the conjunctiva (timolol gellan) on the 24-h heart rate of patients currently being treated for glaucoma to quantify the reduction in mean heart rate. Both timolol gel and timolol solution modestly decreased the 24-h heart rate compared with placebo [104]. Both reductions in mean 24-h heart rate were similar and most pronounced during the active, day-time period [104].

With the same aim and using the methodology, Balasubramaniam *et al.* [10] developed an ion-activated *in situ* gelling system of indomethacin (a nonsteroidal anti-inflammatory drug used as an alternative to steroids in the treatment of uveitis and other external inflammations of the eye). In this study, Gelrite® gellan gum was used at a concentration between 0.1 and 0.5% (w/w) formulated with indomethacin 1% (w/w) [10]. Increasing the concentration beyond 0.5% caused gelation upon cooling to 40 °C [10]. The formulations exhibited pseudoplastic rheology and the *in vitro* release studies showed that the formed gels had the ability to retain indomethacin for the duration of the study (eight hours). The formulations demonstrated better therapeutic efficacy compared with the standard suspension because they were successful in improving the clinical parameters monitored for prolonged periods (24 hours) [10].

Tayel *et al.* [105] developed alternative controlled-release *in situ* ocular drug-loaded nanoemulsion (NE) gels based on gellan gum formulated with terbinafine hydrochloride. This NE was prepared using oils (isopropyl myristate/Miglyol® 812), surfactants (Tween® 80/Cremophor® EL), a co-surfactant [polyethylene glycol (PEG) 400] water and thereafter dispersed in a gellan gum solution with a fixed concentration of 0.2%(w/w). The formulation with Miglyol® 812, Cremophor® EL: PEG 400 (1:2) and water (5, 55 and 40%, w/w, respectively) showedbetter results in that the gels were thermodynamically stable, transparent, pseudoplastic, mucoadhesive and showed more retarded zero-order drug-release rates [105].

Alginates have a long history of use in numerous biomedical applications, including drug delivery systems, because they are biodegradable, biocompatible and mucoadhesive polymers [36]. Ophthalmic formulations containing alginic acid have the ability to gelate in the eye and have mucoadhesive properties [6,36]. Alginic acid is a linear and anionic block copolymer polysaccharide comprising β-D-mannuronic acid and α-L-glucuronic acid residues joined by 1,4-glycosidic linkages [6]. Dilute aqueous solutions of alginates form firm gels on addition of di- and trivalent metal ions by a cooperative process involving consecutive glucuronic residues in the α-L-glucuronic acid blocks of the alginate chain [6]. Generally, this polymer is used in combination with other polymers; Table 1 details examples of its use in ophthalmic formulations.

Ophthalmic formulations with two or more stimuli-responsive polymers

The preparation of ophthalmic pharmaceutical formulations with different *in situ* gelling polymers that have different stimuli-responsiveness mechanisms that explore the unique physicochemical characteristics of the ocular tissues is an excellent strategy that has yet to be tested [3]. Thereby, over recent years, the combination of thermo-responsive polymers, pH-sensitive polymers or ion-activated polymers in the same ophthalmic formulation has been investigated. Recently, the formulation of these biodegradable polymers as colloidal carriers systems, such as polymeric micelles, nanosuspensions or lipid-based nanocarriers, has proven to be the most effective strategy, causing an exponential increase in the bioavailability of the ophthalmic drugs [1,4,8,14–17]. Table 1 shows some examples of the use of two or more stimuli-responsive polymers in ophthalmic pharmaceutical systems.

Concluding remarks

As can be seen from the studies discussed in this review, the preparation of ophthalmic pharmaceutical formulations with different *in situ* gelling polymers that have different stimuli-responsiveness mechanisms that explore the unique physicochemical characteristics of the ocular tissues is an excellent strategy that requires further investigation. In fact, the combination of two or more stimuli-responsive polymers in the same formulation holds promise for greater compliance and improved therapeutic efficacy, because it takes advantage of the intrinsic characteristics of the ocular globe, increasing the retention time and, therefore, the bioavailability of the drug used. Over recent years, the use of such biodegradable and biocompatible polymers in colloidal carrier systems has proved to be the most effective strategy (e.g. hydrogels, polymeric micelles, nanosuspensions and lipid-based nanocarriers). These kinds of strategy offer many advantages as delivery systems for ophthalmic administration, such as improving the bioavailability of poorly soluble drugs, administration of a precise dosage, targeted and good controlled release properties and the reduction of adverse effects.

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