EXPERT OPINION

In situ gel systems as 'smart' carriers for sustained ocular drug delivery

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Introduction: *In situ* gel systems refer to a class of novel delivery vehicles, composed of natural, semisynthetic or synthetic polymers, which present the unique property of sol–gel conversion on receipt of biological stimulus.

Areas covered: The present review summarizes the latest developments in *in situ* gel technology, with regard to ophthalmic drug delivery. Starting with the mechanism of ocular absorption, the review expands on the fabrication of various polymeric *in situ* gel systems, made up of two or more polymers presenting multi-stimuli sensitivity, coupled with other interesting features, such as bio-adhesion, enhanced penetration or sustained release. Various key issues and challenges in this area have been addressed and critically analyzed.

Expert opinion: The advent of *in situ* gel systems has inaugurated a new transom for 'smart' ocular delivery. By virtue of possessing stimuli-responsive phase transition properties, these systems can easily be administered into the eye, similar to normal eye drops. Their unique gelling properties endow them with special features, such as prolonged retention at the site of administration, followed by sustained drug release. Despite the superiority of these systems as compared with conventional ophthalmic formulations, further investigations are necessary to address the toxicity issues, so as to minimize regulatory hurdles during commercialization.

Keywords: *in situ* gel systems, ocular drug delivery, polymers, sol–gel interconversion, stimuli-responsive

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1. Introduction

Drug delivery refers to the science and engineering of transforming pharmaceutically active molecules or bioactives into practically implementable therapeutic modalities. The most critical challenge of today's health care is the timely liberation of therapeutics to their specific target in a safe, reproducible and patient-compliant manner. As for conventional therapy is concerned, regardless of the route of administration, drug molecules on their way from the point of administration to their pathological target are continuously challenged by multiple physiological barriers such as enzymatic degradation of drug molecule in the stomach, absorption across intestinal epithelium, hepatic clearance, short plasma half-life and nonspecific tissue distribution. These barriers, albeit crucial for the maintenance of vital physiological functions of our body, the therapeutic efficacy of the administered drugs is compromised to a considerable extent. The primary challenges in the field of drug delivery thus reside in complete understanding of these barriers and develop novel strategies to circumvent them.

In traditional therapy, a variety of routes are exercised for administering drugs to patients. These include but not limited to the noninvasive peroral, topical,

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Article highlights.

- *In situ* gel systems refer to a class of novel delivery vehicles, which present unique property of sol–gel phase transition on receipt of biological stimulus.
- *In situ* gels can effectively improve the retention time and prevent rapid drainage of instilled drug from the ocular site.
- The sensitivity against the biological stimulus can be improved by using a combination of polymers that respond to multiple biological stimuli.
- Phase transition property endows the system with sustained-release properties, which opens a new transom for the treatment of ocular disorders where a sustained drug release is required.
- Formulation engineering is easy to achieve as interesting features *viz* bio-adhesion or improved penetration can be applied by combining these systems with other polymers.

This box summarizes key points contained in the article.

transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation. Needless to mention, human eyes are one of the most delicate and crucial sense organ associated with vision. Among the various delivery routes, ophthalmic/ocular drug delivery is one of the most interesting and challenging endeavors that is currently being faced by the pharmaceutical scientists. As an isolate organ, eye is quite difficult to study from the drug delivery point of view. Yet the advantage of ocular route can be easily valued as the drug enters the systemic circulation while successfully bypassing the effect of hepatic first pass.

A meticulous look toward the past of ocular delivery clearly accentuates eye drops as the principal and most frequently used formulation for ocular delivery. Although eye drops are easy to manufacture and patient compliant, their application is severely fraught with problems related to their poor bioavailability (BA, 1 – 10%) [1]. Poor BA through ocular route may be accredited to limited area of absorption, tight junction of the superficial conjunctival epithelium, pre-systemic metabolism of drugs in the ocular milieu and nonspecific binding with lachrymal proteins [2]. Another factor that contributes equally to poor BA is the rapid drainage of instilled dose from the site of application. Rapid drainage is associated with natural tendency of eye to maintain the residence volume at 7 – 10 µl [3], which is much less than the normal volume instilled at once, that is, 20 – 50 µl. Rapid drainage is contributed by high turnover of lacrimation, which result in very less residence time for drugs to be biologically available. Issues pertinent to less residence time were taken into account, and in course of future, ointment and suspension formulations came into picture. The ointment formulations were introduced with the concept of increasing viscosity, which in turn was anticipated to prolong the residence time. These formulations not only circumvented the limitations associated with rapid drainage of drug from the eyes but also exhibited higher retention at the site of application for a protracted time period. However, blurred vision led to patient incompliance [4] and restricted their usages during night hours only. Development of suspension dosage form was based on the fact that particulate formulations might present better retention in cul-de-sac. Compared with solutions, suspensions exhibited better activity in terms of their prolonged retentivity. However, results are often variable due to sedimentation on storage and improper shaking before use. Over the last decade, researchers are continuously striving to circumvent the drawbacks associated with the conventional ophthalmic formulations. Consequently, a series of new approaches such as vesicular systems, nanoparticulate systems, hydrogels, implantable systems, collagen shields and ocuserts have come to the fore and have become subjects of intensive investigations. These new delivery systems were ornamented with a variety of approaches having advantages of increased residence time, controlled and sustained drug release over a longer period, reduction of side effects and better patient compliance in comparison with the conventional formulation for ocular delivery. These include but not limited to the

- use of different polymeric combinations to provide bio-adhesion and controlled release in nanoparticulate systems and use of penetration enhancers in hydrogels and nanoparticles and [5]
- use of positively charged liposome to obtain improved bio-adhesion [6].

Although these systems have been reported with successful deliverability up to some extent, they are not free from pitfalls like

- poor patient compliance,
- need of minor surgery in implantable systems,
- difficulty in self-insertion,
- corneal epithelial disruption by some of polymers such as poly (alkyl cyanoacrylate), [7]
- premature release of drug,
- corneal epithelial surface disruption due to penetration enhancer,
- toxicological complications,
- irritation due to surfactants and bile salts and
- instability of the formulation.

In view of the above pitfalls, an ideal formulation would be the one which is comparable with conventional eye drops in terms of their ease of administration and prolonged retentivity at the site of application comparable with novel approaches. To be more precise, the ideal formulation is one that can effectively oppose its clearance from the target site due to nasolacrimal drainage and thereby result in an improved BA, reduced dosing frequency and of course improved patient acceptability. In such a scientific panorama, emergence of *in situ* gel system promises to circumvent many of the drawbacks associated with

conventional ophthalmic formulations and open new vistas for 'smart' ocular delivery.

Two systematic reviews, covering various aspects of *in situ* gel-based ocular delivery systems, have been published in 2007 and 2009 respectively [8,9]. As these reviews were published sometimes back, the bibliographies covered are not up-to-date and mostly include secondary literatures published in between 1990 and 2005. As expected, in the last 5 – 7 years, a variety of novel *in situ* gelling systems have come to the fore; many of these systems have been examined in terms of their ocular deliverability. With the advent of new polymeric systems and emergence of newer strategies for ocular BA enhancement, the field has marched at a phenomenal pace, necessitating recompilation and reanalysis of the up-to-date advancements in germane area. The present review, therefore, examines the recent developments in *in situ* gel technology with regard to ophthalmic drug delivery. Starting with the mechanism of ocular absorption, the review extends its depth and breadth into the various polymeric *in situ* gel systems that are currently being investigated for ophthalmic applications. Selection and use of various polymers as *in situ* gel components and their biomedical applicability have been considered as a part of our discussion. Various key issues and challenges have been critically analyzed and addressed. Thus the prime target of the present account is to convey information about the state-of-the-art pertinent to the application of *in situ* gel systems and critically address the limitations and need for further progress.

2. Ocular penetration

The topical delivery through ocular route has been extensively used for the local treatment of eye pathologies. Poor BA pertinent to conventional eye drops is attributed to physiological constraints such as limited area of absorption, lipophilic temperament of the corneal epithelium and a series of elimination factors such as nasolacrimal drainage, tear turnover and tear evaporation that reduce the contact time of medication with the corneal surface. A schematic model, depicting the transportation and fate of drug molecule with the challenges, is shown in **Figure 1**.

Precorneal tear film is the first barrier encountered by the drug to be absorbed. It consists of three layers: (i) the outer most layer (comprised of oil and lipid, which prevents tear evaporation); (ii) the middle layer (aqueous salt solution layer) and (iii) the inner most layer (mucous layer). Ocular membranes comprise cornea, which is nonvascularized, and the conjunctiva, the vascularized one. The corneal epithelium comprises five or six layers of non-keratinized squamous cells, and it is considered to be the major pathway for ocular drug penetration. The major challenge of ocular delivery is associated with the elimination of instilled dose by a number of elimination factors (**Figure 1**), which can be resolved effectively by formulation engineering. Development of formulation with improved retention time is the only solution to this problem.

3. *In situ* gel system

As already stated, a variety of novel drug delivery systems have been experimented for ocular delivery in the last few years. Among all these systems, *in situ* gels deserve special mention as they provide a lucrative approach for ocular drug delivery, which is comparable with both conventional and novel approaches for ocular delivery. *In situ* gel systems comprise delivery vehicles composed of the polymers (natural, semisynthetic or synthetic) with unique property of sol–gel conversion when influenced by biological stimulus. This inimitable property of sol to gel conversion provide various advantages to these systems such as

- easy administration like a conventional eye drop formulation,
- reproducible and accurate dosing,
- ease of fabrication,
- prolonged retentivity at the site of action,
- sustained drug release due to gel network formed after being influenced by the physiological stimulation
- easy scale-up and sterilization and
- ease of system engineering in a combinatory approach (by choosing polymers with multiple function penetration enhancer/mucoadhesion/*in situ* gel property).

In view of the above advantages, *in situ* gel systems with unique properties can be designated as system of choice in ocular drug delivery. *In situ* gel systems show phase transition from sol to gel upon getting biological stimulus. Three types of biological stimulus are presented by ocular route *viz* temperature, pH and ions, present in the lachrymal fluid. Although a number of polymeric combinations with different stimuli-sensitivity profiles have been reported, in-depth discussion about all the combinations is beyond the scope of our discussion. Interested readers may consult some earlier reviews for more detailed, comprehensive discussion [10-12].

3.1 Temperature-sensitive gelling system

Temperature-sensitive *in situ* gelling systems are probably the most commonly studied class of stimuli-sensitive polymer systems in drug delivery research [13]. These systems respond to change in temperature as an external stimulus. The temperature at which sol–gel transition occurs is referred as the lower critical solution temperature (LCST). The difference in solubility at different temperature is assumed to be the main reason for sol to gel conversion. At temperature below the LCST, hydrogen bonding between the hydrophilic groups on polymeric surface and water molecule favors enhanced dissolution of the polymer chains and the system remains in the form of solution. As the system is placed at temperature greater than LCST, the hydrogen bonds corrupt. Consequently, the hydrophobic interaction is increased, thereby facilitating sol–gel transformation [14]. Readers interested in in-depth understanding of temperature-sensitive

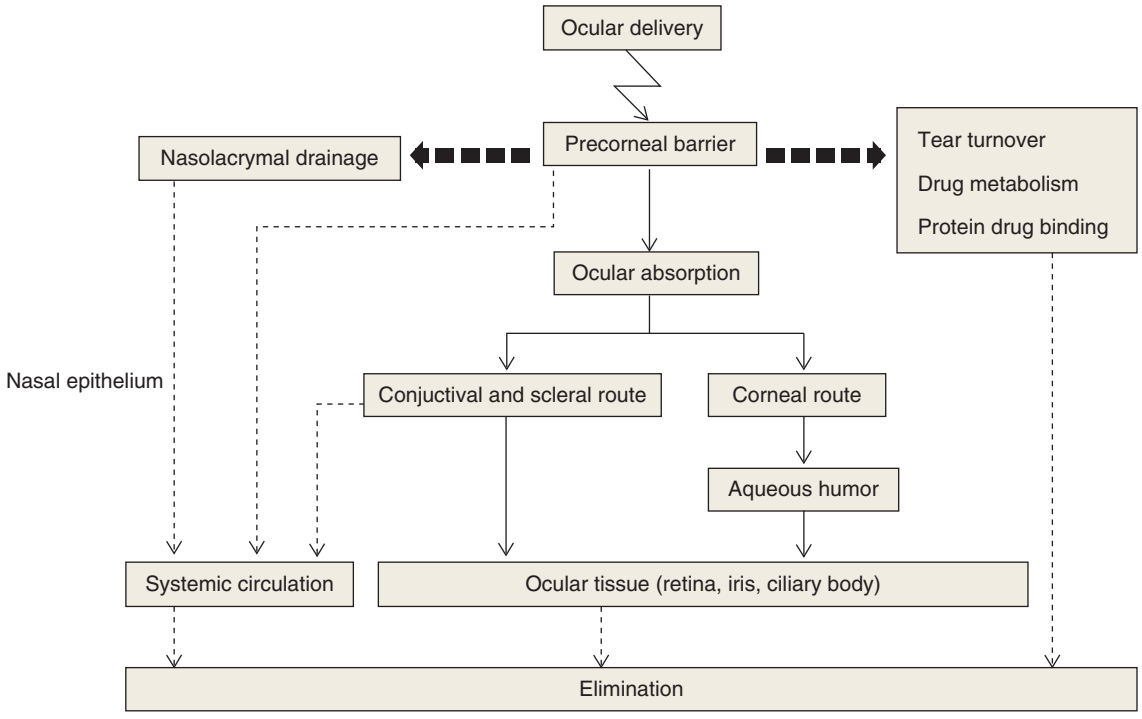

Figure 1. Schematic model depicting the drug movement and barriers in ocular delivery.

in situ gel system are directed to excellent reviews published earlier [15,16].

3.1.1 *Natural polymers and derivative*

Human being has been blessed by a number of gifts from Mother Nature. Polymers with *in situ* gelling properties are one of them. These polymers, either alone or in combination with others, have been used in the fabrication of novel *in situ* gel system with desirable properties.

3.1.1.1 *Cellulose derivatives*

Cellulose is a water-insoluble polysaccharide, consisting of a linear chain of several hundred to more than ten thousand β (1 4)-linked D-glucose units. Alkylation of cellulose results in the formation of cellulose derivatives, which show *in situ* gelling properties at low concentration (1 – 10%). Methyl cellulose (MC) (**Figure 2A**) and hydroxypropylmethylcellulose (HPMC) (**Figure 2B**) are typical examples of these cellulose derivatives.

The phase transition temperatures for these polymers fall within the range of 40 – 50°C and 75 – 90°C for MC and HPMC respectively, which make them unsuitable in terms of ocular deliverability. Certain physical and chemical modifications such as addition of NaCl lowers the phase transition temp of MC up to 32 – 34°C [17]. A detailed study concerning the effect of various salts on gelation and drug release was very recently reported [18]. Addition of sodium chloride (5 – 7% w/v), potassium chloride (8 – 9% w/v) or sodium bicarbonate (5% w/v) reduced the gelation temperature of MC (1% w/v) from 60 to below 37°C. Addition of salts also prolonged the duration of drug release from 1.5 to 3 – 5 h. Although addition of different salt solutions lowered the phase transition temperature below the body temperature, the practical utility of the formulation is still questionable. Salts added in such high concentrations may disrupt the iso-osmolarity of the formulation, which, in turn, is detrimental to ocular tissue. Further investigations in this regard are necessary to elucidate the effect of such higher concentration on the iso-osmolarity of the final formulation. At higher temperature, polymer–polymer interaction becomes dominant, which is supposed to be the mechanism of phase transition at higher temperature [16]. It should, however, be mentioned that cellulose derivatives, alone, have not been used so much in ocular delivery. However, their usages in combination with other *in situ* gelling polymers have been reported to enhance the effectiveness of the cocktail recipe. Carboxy methyl cellulose (CMC), MC and HPMC have been used as viscosity enhancers in combination with Pluronic F127 and are reported with improved performance in terms of not only increased viscosity but also sustained release in comparison with Pluronic F127 alone [19-21]. In further course of research, MC has been used in combination with Carbopol and reported to increase the viscosity of the proposed system. MC (1.5%) in combination with Carbopol (0.3%) resulted in a formulation with low viscosity, which formed a strong gel under simulated physiological conditions [22]. From the above discussion, it follows that increase in viscosity will fortify the

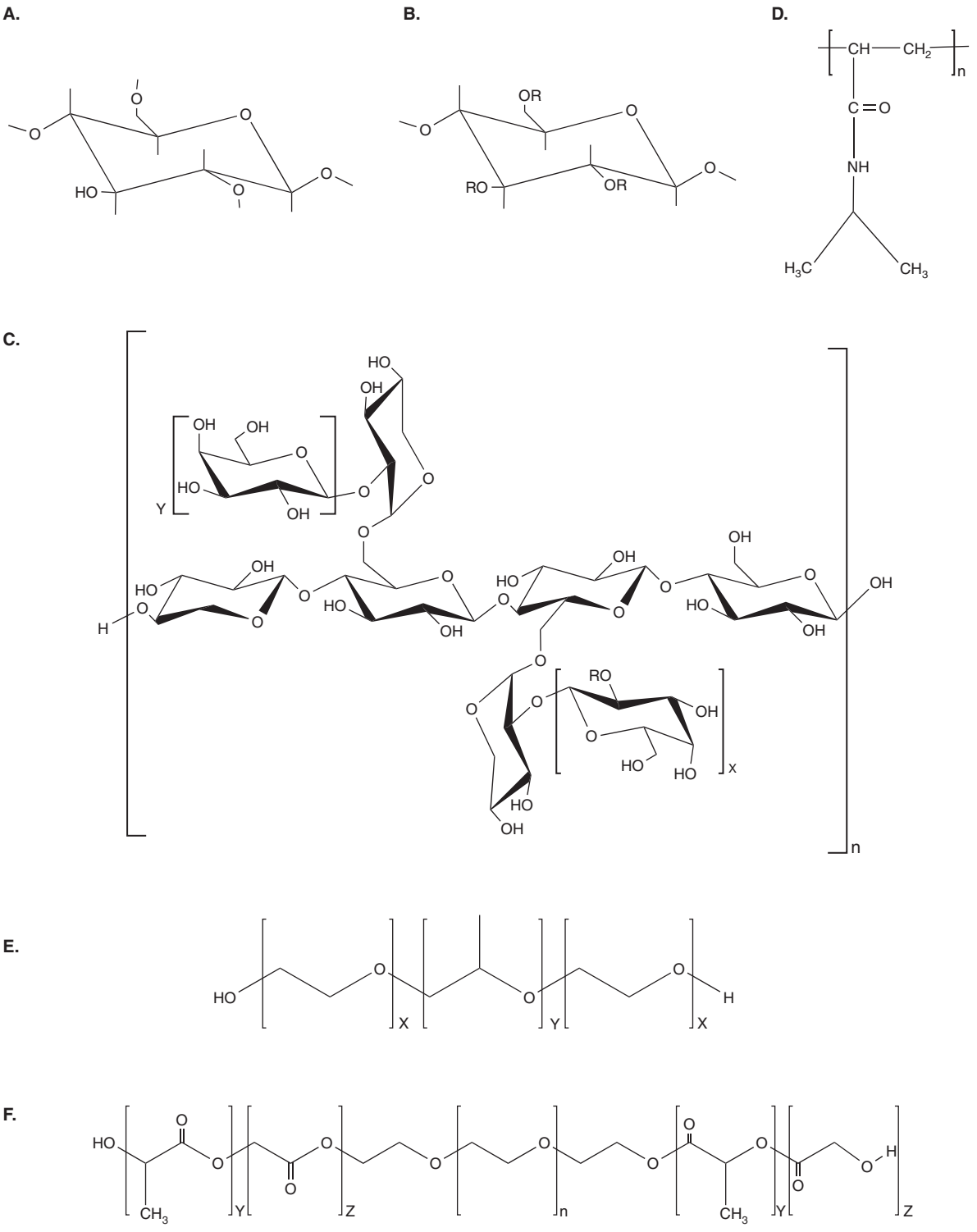

Figure 2. Structure of (**A**) MC, (**B**) HPMC where R = H or CH_3 or $CH_2CH(OH)CH_3$, (**C**) Xyloglucan, (**D**) PNIPAAm, (**E**) Poloxamer, (**F**) PLGA–PEG–PLGA tri-block copolymer, (**G**) Chitosan, (**H**) Carbomer, (**I**) Gellan gum and (**J**) Alginate (i) and (ii) monomers (iii) chain conformation

HPMC: Hydroxypropylmethylcellulose; MC: Methyl cellulose; PLGA: Poly-(DL-lactic acid-co-glycolic acid); PEG: Polyethylene glycol.

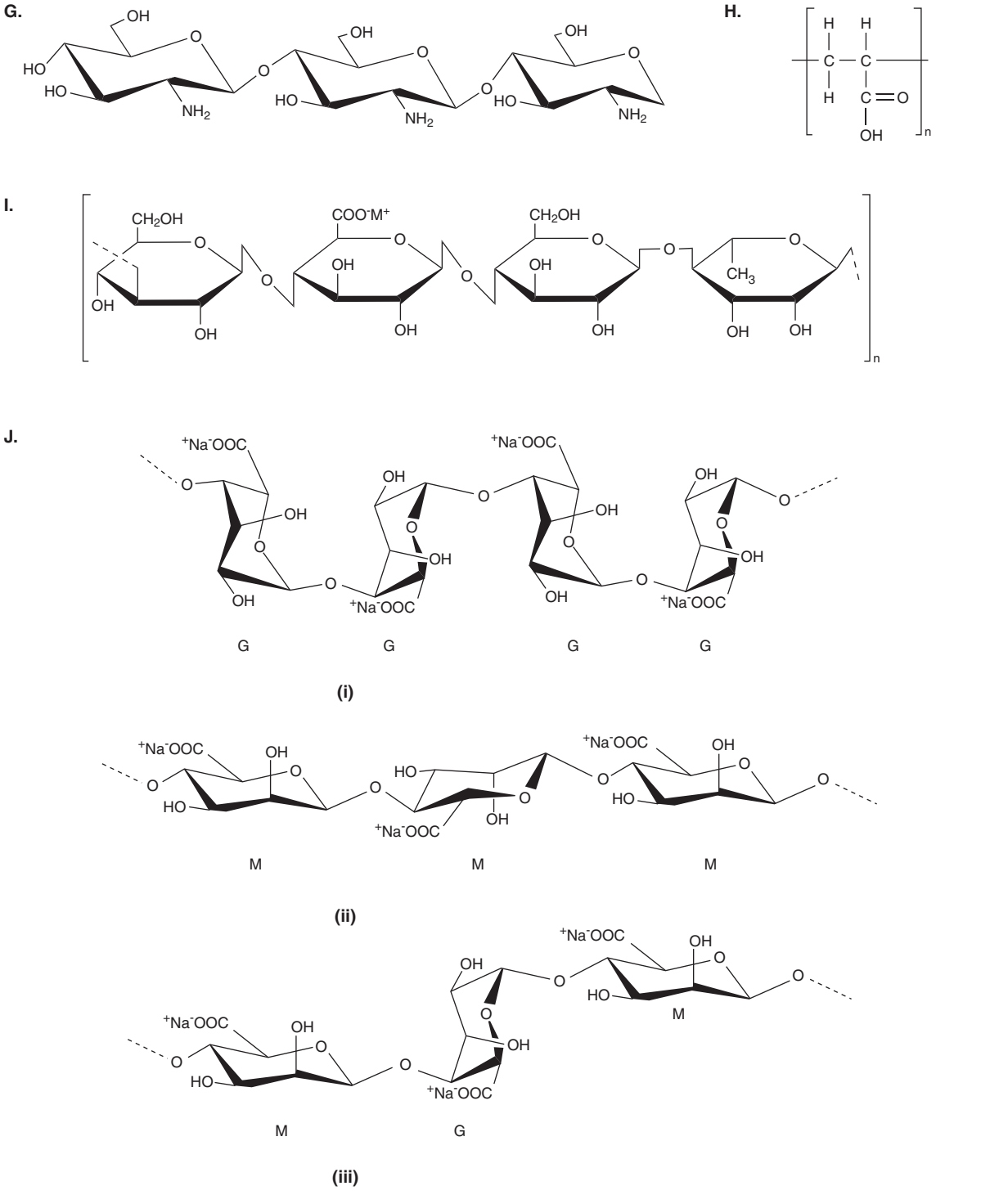

Figure 2. Structure of (**(continued).**) MC, (**B**) HPMC where R = H or CH_3 or $CH_2CH(OH)CH_3$, (**C**) Xyloglucan, (**D**) PNIPAAm, (**E**) Poloxamer, (**F**) PLGA–PEG–PLGA tri-block copolymer, (**G**) Chitosan, (**H**) Carbomer, (**I**) Gellan gum and (**J**) Alginate (i) and (ii) monomers (iii) chain conformation

HPMC: Hydroxypropylmethylcellulose; MC: Methyl cellulose; PLGA: Poly-(DL-lactic acid-co-glycolic acid); PEG: Polyethylene glycol.

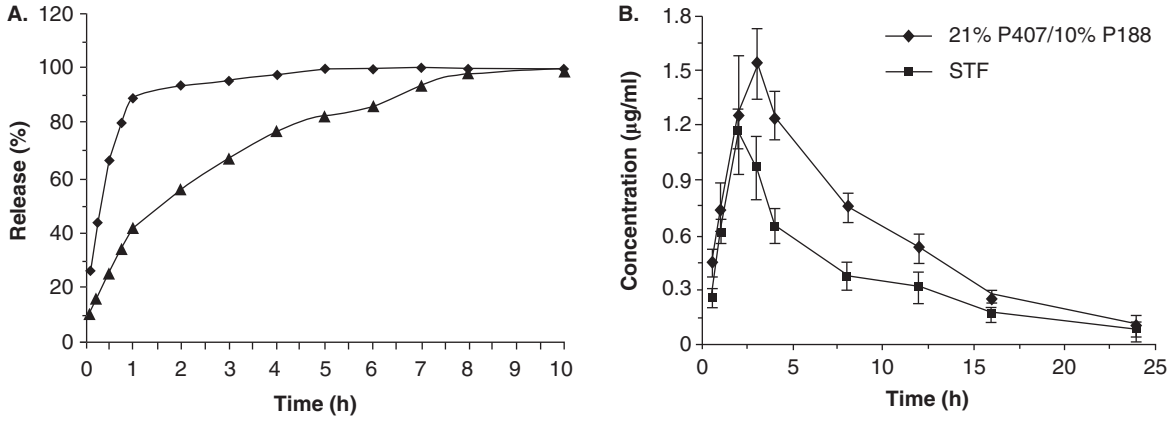

Figure 3. (**A**) Cumulative amount of methazolamide (MTA) released from Poloxamer formulation (▲) and MTA in simulate tear fluid (STF) (◆). (**B**) MTA concentration in rabbit aqueous humor from Poloxamer formulation (◆) and MTA-containing STF (■).
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therapeutic effect by prolonging the retention time. In order to determine the effective range of viscosity, the effect of viscosity (10 – 100 mPa.s) on clearance from the precorneal surface was studied by Zaki and coworkers. The authors reported that the retention began to increase only after the viscosity exceeded a critical value of 10 mPa.s [23]. Although increased viscosity leads to protracted retention, the same might also cause discomfort and damage to ocular epithelia due to an increase in the shear stresses during blinking. A mathematical model was developed by Zhu and Chauhan for quantitative determination of the effect of viscosity on drainage of the instilled dose. Formulations with viscosity of around 100 mPa.s were best suited for this purpose. Increased viscosity not only prolongs the retention time but also ensures safety. Viscous formulations hardly cause damage to ocular epithelia due to excessive shear during blinking [24].

3.1.1.2 *Xyloglucan*

Xyloglucan is a natural polymer, derived from tamarind seeds and composed of a backbone of β-(1→4)-glucose residues with side chains of α-(1→6)-xylose partially substituted with β-(1→2)-galactoxylose. This polymer, being composed of three units of xyloglucan oligomers with heptasaccharide, octasaccharide and nonasaccharide, possesses different number of galactose side chains (**Figure 2C**).

It hardly shows any phase transition property. Yet partial degradation of this polymer in presence of β-galactosidase imparts thermally induced *in situ* gelation property. This thermal sensitivity is imparted only when galactose removal ratio exceeds by 35%. Thermally induced sol–gel transition of xyloglucan has been shown to decrease 40 to 5°C upon increasing the galactose removal ratio from 35 to 58%.

Xyloglucan formulation (2% w/w) was assessed for ocular delivery of timolol using Droptimol® (eye drop of timolol) and Timoptic® XE (gellan gum-based *in situ* gel formulation) as positive control. The developed formulation was assessed for the drug concentration in tear fluid, cornea, iris–ciliary body, aqueous humor and plasma and comparative reduction in intraocular pressure. Xyloglucan gel showed much better profile in comparison with Droptimol while comparable profile with Timoptic XE was obtained [25]. In a comparative study, xyloglucan gel (1.5% w/w) was compared with Pluronic F127 (25% w/w) for the delivery of pilocarpine hydrochloride and the results of the study were really exciting. The xyloglucan gels not only exhibited sustained release but were also similar to Pluronic F127 gels in the degree of enhancement of mitotic response [26]. The most important outcome of the work is that if similar response can be obtained with low concentration of natural biodegradable polysaccharide, then there is no rationale to switch for a synthetic polymer with higher concentration. As the polymer concentration used to develop the formulation is very low (only 1 – 2% w/w), this approach might obviate one more issue on toxicity associated with high concentration of polymers.

3.1.2 *Synthetic polymers*

Although a large number of synthetic polymers have been fabricated with desirable thermosensitive *in situ* gel properties, the discussion will be restricted to derivatives used in ocular delivery. Readers, interested for in-depth understanding are directed elsewhere [16,17].

3.1.2.1 *N*-Isopropylacrylamide-based derivatives

N-Isopropylacrylamide (NIPAAm) (**Figure 2D**)-based homopolymer and its copolymers have been investigated extensively in drug delivery. An aqueous solution of NIPAAm precipitates above its LCST (32°C). Temperature below the LCST leads to dominant hydrogen bond between water molecule and polymer resulting in dissolution of the polymer chain. Above LCST, water molecules escape from the surrounding

Table 1. List of temperature-sensitive *in situ* gelling system explored in ophthalmic drug delivery.

Ingredients/polymers	Drug	Strategy used	Key findings	Ref.
Pluronic F127, MC, HPMC, CMC	Timolol maleate (TM)	Increased viscosity by addition of cellulose derivatives	2.4-fold increased bioavailability	[19]
Pluronic F127, PEG, PVP, PVA, MC, HPMC	Pilocarpine hydrochloride	Increased viscosity by addition of cellulose derivatives	Controlled release when used in combination with MC and HPMC	[21]
Xyloglucan	Timolol maleate	Temperature-induced gelation	1.8-fold AUC in iris–ciliary body compared with conventional eye drop 1.61-fold higher C_{max} in aqueous humor in comparison with conventional eye drop	[25]
Xyloglucan	Pilocarpine hydrochloride	Temperature-induced gelation	Xyloglucan (1.5%) was similar to Pluronic F127 (25%) in degree of enhancement of mitotic response	[26]
PNIPAAm	Epinephrine	Temperature-induced gelation	Six times decrease in IOP in comparison with eye drop	[27]
PNIPAAm-g-PHEMA	Epinephrine	Temperature-induced gelation	Reduction in IOP up to 26 h when compared with simple eye drop (IOP lowering up to 8 h)	[28]
PNIPAAm–Chitosan	Timolol maleate	Temperature-induced gelation	Twofold higher C_{max} Reduction of (IOP) up to 12 h Less toxic	[29]
Poloxamer 407	Pilocarpine hydrochloride	Temperature-induced gelation	1.9-fold increase in mitotic response for Poloxamer gel than aqueous solution	[30]
Poloxamer 407, Poloxamer 188	rhEGF/HP-β-CD	Temperature-induced gelation	1.6- to 1.8-fold AUC of Poloxamer gel containing the rhEGF/HP-β-CD complex than that containing rhEGF solution	[32]
Poloxamer 407, Poloxamer 188	Methazolamide (MTA)	Temperature-induced gelation	2.29-fold AUC in comparison with drug in STF IOP-lowering effect up to 12 h	[34]
Pluronic F127-g-poly (acrylic acid)	Gatifloxacin (GTX)	Temperature-induced gelation	Drug resident time and the total resident amount in rabbits' conjunctival sac increased by 5.0- and 2.6-folds for in situ gel, compared with eye drops	[69]
Pluronic-g-poly(acrylic acid)	Vit-B_{12}	Temperature-induced gelation	Drug resident time and the total resident amount increased by 4-fold and 1.2-fold for *in situ* gel compared with eye drops	[70]
MC, fructose, sodium citrate (SC)	Ketorolac tromethamine (KT)	Temperature-induced gelation	Addition of fructose and sodium citrate reduce the gelation temperature from 59 to 32°C Sustained release up to 9 h	[71]
MC, HPMC	Ketorolac tromethamine (KT)	Temperature-induced gelation	Sustained release up to 4 h	[72]
Pluronic F127, Pluronic F68 and sodium hyaluronate	-	Temperature-induced gelation in addition to mucoadhesive property	Threefold increase in corneal residence time No additive effect of sodium hyaluronate on corneal retention	[73]
Pluronic F127, Poloxamer 188, HPMC, HEC	Ciprofloxacin hydrochloride	Temperature-induced gelation in addition to mucoadhesive property	P407/P188/HPMC (18/13/1.5%, w/w), and P407/P188/HEC (18/13/0.5%, w/w) showed optimum Mucoadhesion Sustained drug release up to 8 h	[74]

Table 1. List of temperature-sensitive *in situ* gelling system explored in ophthalmic drug delivery (continued).

Ingredients/polymers	Drug	Strategy used	Key findings	Ref.
Pluronic F127, Pluronic F68, hyaluronic acid (HA)	Acyclovir	Temperature-induced gelation in addition to mucoadhesive property	Rheological synergism between poloxamers/HA gel Prolonged drug release up to 6 h	[75]
Pluronic F127, Poloxamer 188, Xanthan gum, sodium alginate	Moxifloxacin HCl	Temperature-induced gelation in addition to mucoadhesive property	Combination of polymers was better in comparison with polymers alone	[76]
Pluronic F127, Pluronic F68, Sodium hyaluronate	Sparfloxacin	Temperature-induced gelation in addition to mucoadhesive property	Sustained release up to 24 h	[77]

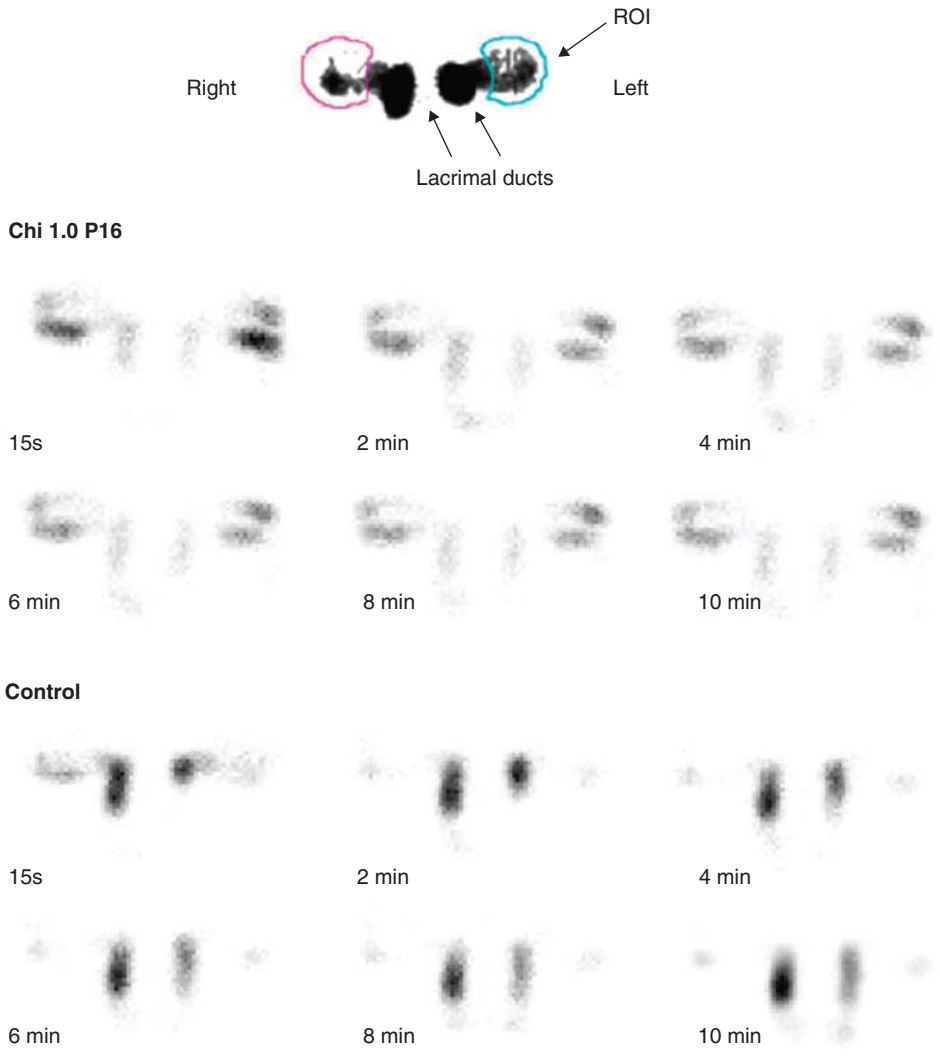

Figure 4. Scintigraphic images of eye up to 10 min with developed formulation (Chitosan 1% w/v Poloxamer 16% w/v) and saline control.

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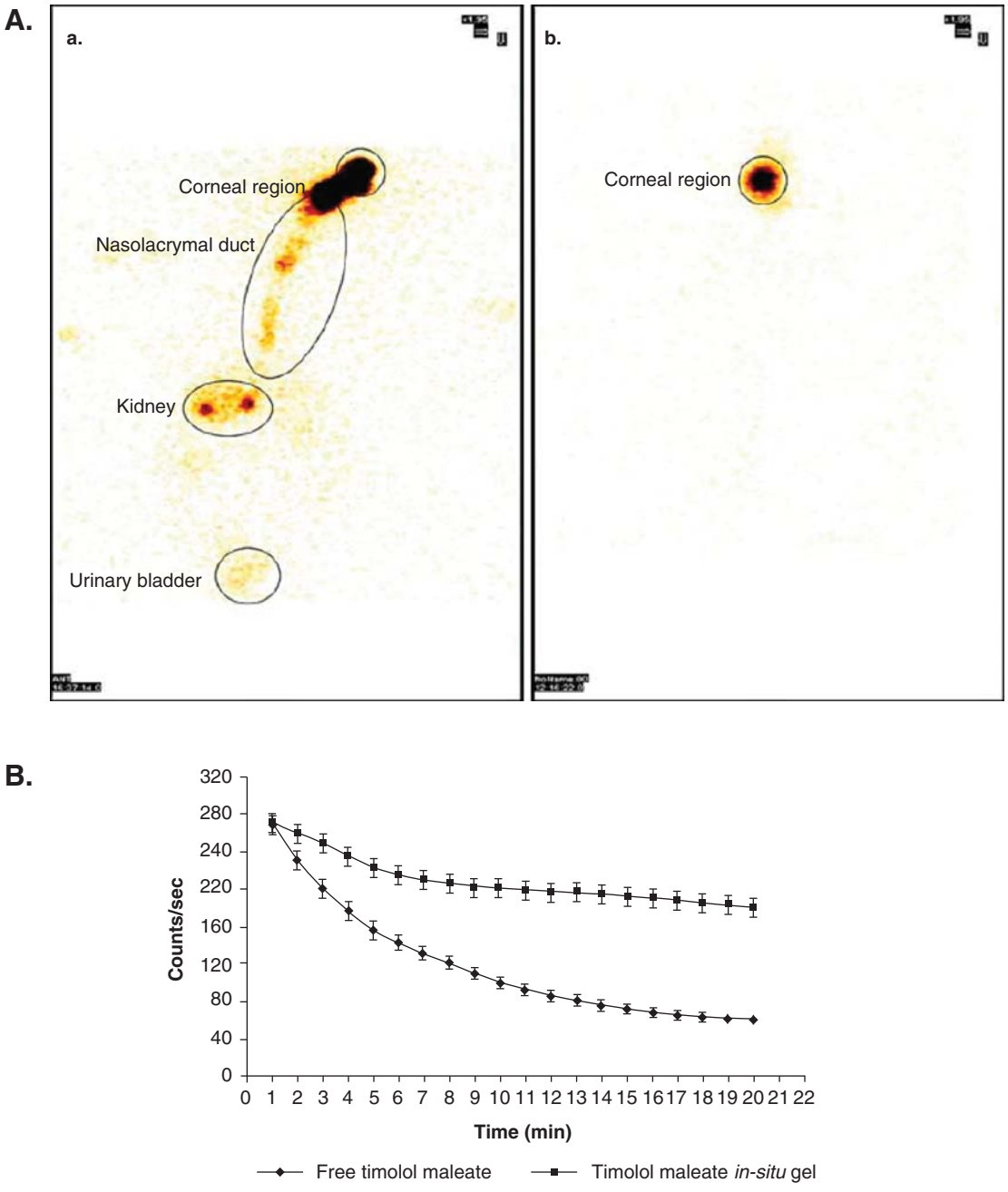

Figure 5. (A) Whole-body image after 2 h of administration: (a) Plain drug solution and (b) *In situ* gel system. **(B)** Time–activity curve shows precorneal drainage.
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polymeric chain, resulting in dominant hydrophobic attraction and gel formation. Poly-*N*-isopropylacrylamide-based controlled release formulation of epinephrine for glaucoma treatment was developed [27]. Linear PNIPAAm and cross-linked PNIPAAm nanoparticles were developed and evaluated in terms of their cytotoxicity and intraocular pressure (IOP)-lowering effect in rabbits. Results indicated that the formulations not only are non-cytotoxic but also reduced the IOP six and eight times by linear PNIPAAm and cross-linked nanoparticles respectively when compared with simple eye drops. Poly (*N*-isopropylacrylamide-g-2-hydroxyethyl methacrylate) (PNIPAAm-g-PHEMA)-based *in situ* gel system was further investigated by the same group and reported to reduce IOP up to 26 h when compared with simple eye drop (IOP lowering up to 8 h). Progressive release was observed due to entrapment of drug within the tangled polymer chains. PHEMA macromer contents and cross-linking density were reported to be the main contributing

factors in progressive drug release. Finally PNIPAAm-based formulation was concluded as an effective alternative of conventional eye drop formulation [28].

In another combination, PNIPAAm has been combined with chitosan (PNIPAAm–CS) [29] to form a thermosensitive gel-forming system using timolol maleate as the model drug. The LCST for the proposed system was measured to be 32°C, suggesting the thermosensitive potential of the proposed system. Twofold higher C_{max} and stronger capacity to reduce IOP up to 12 h in comparison with conventional eye drops were the key findings of the report. The presented combination was not only pharmacokinetically superior but simultaneously non-cytotoxic in concentration range of 0.5 – 400 µg/ml.

3.1.2.2 PEO/PPO-based system (Poloxamers)

These polymers are non-ionic ABA-type triblock copolymers composed of a central hydrophobic chain of poly (propylene oxide) flanked by two hydrophilic chains of poly (ethylene oxide) (PEO-PPO-PEO) (**Figure 2E**). These are more popular by their trade name Pluronics. Poloxamers are available in different grades depending on the ratio of the block with different gelation properties. Among the different grades available, Pluronic F127 is the most extensively studied polymer in pharmaceutical technology. Starting from the first recognition that concentrated aqueous solutions of Poloxamer can form thermoreversible gels, Poloxamer has traveled a long distance. Significant efforts have been devoted to elucidate the exact mechanism of sol–gel interconversion. A change in micellar properties as a function of concentration and temperature may be a possible cause of such interconversion. Micellar mode of association of aqueous Poloxamer solutions was confirmed by ultrasonic velocity, light-scattering and small-angle neutron scattering measurements. Above the critical micelle concentration (CMC), Poloxamer molecules aggregate and form micelles. General CMC value for poloxamers used in pharmaceuticals is 1 µM to 1 mM at 37°C. Micelle formation in poloxamers shows strong temperature dependence. Below critical micellar temperature (CMT), both PEO and PPO blocks are hydrated and PPO presents appreciable solubility in water. However, as the temperature increases, PPO chains become less soluble, resulting in micelle formation [15].

Among the synthetic polymers, poloxamers have been investigated extensively in ocular drug delivery. Enhanced activity of pilocarpine in Poloxamer 407 gels in comparison with simple solution has been reported [30]. Although a number of successful reports are available regarding the use of Poloxamer in ocular drug delivery, it was not found suitable when subjected to rheological evaluation and corneal residence time in human volunteers. The non-suitability of the Poloxamer system may be attributed to strong concentration dependence of the sol–gel transition temperature combined with dilution that occurs into the ocular site [31]. Later, the Poloxamer system was also reported for successful delivery of rhEGF/HP-β-CD complex [32]. In order to further develop and reduce the concentration of poloxamers used, these were tried in combination with other natural and synthetic polymers. Pluronic F127 in combination with cellulose derivatives has been exploited for the delivery of timolol maleate [19]. In another combination approach, poloxamers were blended with mucoadhesive polymer Carbopol to produce thermosensitive *in situ* gel with mucoadhesive property. A controlled release of puerarin up to 8 h was envisaged. *In vivo* evaluation, that is, elimination of puerarin in tear and IOP-lowering effect, suggested that the proposed system had better ability to retain drug than Poloxamer analogs or Carbopol alone [33]. In a recent study by Qian and coworkers, *in situ* gelling system, based on combination of different poloxamers, has been reported for the delivery of methazolamide (MTA). They found Poloxamer 407 in 21%w/w and Poloxamer 188 in 10%w/w, as optimized concentrations for formulation development. In case of eye drops, about 90% of the drug was released within 1 h while sustained release of drug up to 10 h was observed in case of *in situ* gel formulation (**Figure 3A**). Developed formulation was also compared with eye drop for MTA concentration in aqueous humor and exhibited 1.27- and 1.55-fold higher maximum level of MTA in aqueous humor (C_{max}) and time required to reach maximum concentration (T_{max}), respectively (**Figure 3B**) [34].

In order to further explore Poloxamer-based systems, a combination of poloxamers and Carbopol has been very recently explored for the delivery of diclofenac sodium (DS). The effect of different concentrations of poloxamers and Carbopol on various parameters including transparency, pH, rheological behavior, phase transition temperature, gelling capacity, ocular irritation and *in vivo* ophthalmic absorption of DS was studied. An increase in translucency and a decrease in pH were observed with the addition of Carbopol, which was assumed to be the effect of acrylic acid units of carbopols. Based on the observation, Pluronic F127 (20%) in combination with Pluronic F68 (11%) and Carbopol (0.1%) was taken as optimized formulation and was reported to have good gelling capacity. The formulation was also non-irritant to eye with 2.2-fold higher C_{max} in comparison with conventional eye drop formulation [35].

3.1.2.3 PLGA–PEG–PLGA-based system

One more synthetic tri-block polymer poly-(DL-lactic acid-co-glycolic acid) (PLGA)–polyethylene glycol (PEG)–PLGA (**Figure 2F**) with thermosensitive properties has been reported recently as a matrix material for ocular delivery of dexamethasone (DXA) [36].

The polymer in 20%w/w shows LCST at 32°C, which is close to surface temperature of the eye. Sevenfold higher C_{max} of DXA was obtained in comparison with plain drug solution, which suggested the potential application of the newly synthesized system. **Table 1** summarizes temperature-sensitive *in situ* gelling systems that have been explored for ophthalmic applications till date.

Table 2. List of pH-sensitive *in situ* gelling system explored in ophthalmic drug delivery.

Ingredients/polymers	Drug	Strategy used	Key findings	Ref.
Carbopol, MC	_	pH-sensitive system with increased viscosity	Carbopol (0.3%) and MC (1.5%) was found optimum Increased precorneal residence time with enhanced ocular bioavailability	[22]
Chitosan, HPMC	Timolol maleate	pH-sensitive polymer with bio-adhesive property	Clear visible evidence of enhanced retention of *in situ* gel Signified potency of gamma scintigraphy as noninvasive technique	[44]
Carbopol 940, HPMC	Ofloxacin	pH-triggered gelation	Non-irritant Sustained release up to 8 h	[48]
Carbopol 980, HPMC, HP-β-CD	Puerarin	pH-triggered gelation with increased viscosity	1.76-fold T_{max} and 2.17-fold AUC in comparison with drug solution	[49]
Carbopol 940NF, HPMC, Indion 254F	Ciprofloxacin hydrochloride	Reduced occurrence of incompatibility by complexing ciprofloxacin hydrochloride with Indion 254F	Sustained release up to 16 h Formulation was reported to be stable for 2 years	[50]
Carbopol 980NF Na CMC, HP-β-CD	Dexamethasone	Increased drug solubility by complexing it with HP-β-CD	Sustained release with increased bioavailability	[51]
Carbopol 974P, HPMC E4M	Baicalin	pH-triggered gelation with increased viscosity	AUC and C_{max} values were 6.1-fold and 3.6-fold higher as compared with drug solution	[78]
Carbopol® 934, Methocel E15LV	Ketorolac tromethamine	pH-triggered gelation with increased viscosity	About fivefold increase in AUC as compared with eye drop formulation	[79]
Carbopol 940, Chitosan	Timolol maleate	pH-induced gelation	About twofold higher AUC in comparison with eye drop solution	[80]

3.2 pH-sensitive gelling system

pH is another important bioenvironmental parameter present at ocular site and instantaneously forms gel formation upon getting bio-stimulus. The pH sensitivity of these polymers is due to the presence of ionizable groups present on polymer surface, which exhibit a sharp change in degree of ionization and water solubility at specific pH (pKa) [37]. Although a number of natural and synthetic polymers exhibit the pH sensitivity, the present discussion will be restricted to polymers explicitly studied for ocular delivery.

3.2.1 *Natural/Semisynthetic polymers*

Natural polymers with pH-dependent *in situ* gelling property, either alone or in combination with other natural or synthetic polymers, have been explored extensively in ocular drug delivery. Biodegradability and non-toxicity are the two major concerns that make these natural polymers attractive for ophthalmic applications.

3.2.1.1 *Chitosan*

Among the polymers in this category, chitosan has been investigated extensively in ocular drug delivery. Chitosan is a deacetylated product of chitin (**Figure 2G**), well known for its biocompatibility, biodegradability and mucoadhesive properties as well. This polymer has been widely investigated in the fabrication of *in situ* gelling systems.

Chitosan is a cationic polymer that shows pH-dependent solubility; at acidic pH (below its pKa 6.2), it remains as clear solution but at higher pH, that is, at physiological pH, it is converted into soft gel. Favorable properties such as non-toxicity, bio-adhesion and phase transition continue to attract the researcher for investigating this polymer as a key constituent of *in situ* gel systems, either alone or in combination. Chitosan in combination with various polyol salts has also been reported to show temperature-sensitive *in situ* gelling property. In the present discussion, chitosan is categorized as a pH-sensitive polymer, which is based on the combinations reported in literature, so far. Although chitosan-based *in situ* gels have been extensively investigated as injectable [38], nasal delivery [39] and tissue engineering [40] herein, we restrict our discussions explicitly to ocular delivery.

Initially, chitosan-based *in situ* gels were tried alone, but as the discussion of those reports is beyond the scope of the present manuscript, here we tried to incorporate latest advancement in *in situ* gel technology. In our previous work, chitosan-based *in situ* gel was developed in

Table 3. List of ion-activated *in situ* gelling system explored in ophthalmic drug delivery.

Ingredients/polymers	Drug	Strategy used	Key findings	Ref.
Gellan gum	Indomethacin	Ion-induced gelation	Sustained release up to 8 h Improved clinical symptoms of uveitis-induced rabbit	[60]
Gellan gum, Na citrate, Na alginate	Ciprofloxacin hydrochloride	Ion-induced gelation	Sustained release up to 8 h Effective in preventing growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* up to 24 h	[61]
Gellan gum	Perfloxacin mesylate	Ion-induced gelation	Sustained release up to 12 h Formulation reported to be stable for more than 2 years	[62]
Na alginate	Pilocarpine hydrochloride	Ion-activated gelation	Slow release up to 24 h IOP-lowering effect up to 10 h in comparison with simple drug solution (3 h)	[63]
Alginate, HPMC	Gatifloxacin	Ion-activated gelation with increased viscosity	Sustained release with increased retention	[65]
Gellan gum	Moxifloxacin (Mox)	Ion-activated gelation	Sixfold increase in the C_{max} and AUC as compared with marked eye drop	[81]
Gellan gum, Carrageenan	Connexin 43 (Cx43) antisense oligodeoxynucleotide (AsODN)	Ion-activated *in situ* gelling system	Greatest reduction in wound size, the least stromal edema and hypercellularity by *in situ* gel	[82]

combination with Pluronic F127 (timolol maleate as model drug) for exploiting the advantage of dual mechanism of gelation. Chitosan (temperature- and pH-sensitive gelation) in combination with Pluronic (temperature-sensitive gelation) was taken to synergize the effect of and to get the fortified response in terms of gelling sensitivity and penetration-enhancing property of chitosan. The formulation was simple to develop, clear, isotonic and easily convertible to gel above 35°C. Significant higher transcorneal permeation up to 63.41% was observed in comparison with plain drug solution (42.11%), which is attributed to penetration-enhancing property of chitosan. A slow release of 98.03% up to 10 h was observed, which clearly indicated the potential of the developed system for sustained drug release [41].

Later, the combinations of chitosan with different grades of Poloxamer (Pluronic F127 and Poloxamer 188) were studied by other group, taking ciprofloxacin hydrochloride as a model drug. Special emphasis was given to assess the rheological behavior and release kinetics of different combinations. In course, they observed that a combination of Pluronic (15%) and chitosan (0.1%) shows Newtonian flow at non-physiologic conditions (pH 4 and temperature 25°C) while it converts to pseudoplastic at physiologic conditions (pH 7.4 and temperature 37°C). At higher concentration of Pluronic (25%), the combination shows pseudoplastic flow at both physiologic and non-physiologic conditions indicating non-suitability of such higher concentration in fabrication of these systems. High concentration or high molecular weight of chitosan decelerated the release rate by virtue of its decreased penetration through high viscous gel. In addition, the release through all the gel combinations was reported to be Fickian or diffusion mechanism dependent irrespective of the components [42].

This combination was further explored to study the effect of chitosan on texture profile of gel, *in vitro* mucoadhesive strength and scintigraphic studies on human subjects [43]. The texture profile of the gel was measured at different temperatures *viz* 25 and 35°C, in terms of measuring the hardness, compressibility and mucoadhesiveness of the developed formulation. Hardness and compressibility play an important role in removal of formulation from the package and its administration. Lesser the hardness and compressibility of the gel, easier is its removal from the package and subsequent administration into eyes. However, in case the gel is too soft, it will be diluted and drained quickly from the absorption site, which is not desirable. Thus *in situ* gels with optimized hardness would be perfect in performance. As the concentration of chitosan increased from 0.5 to 1.5% w/w, increased hardness and compressibility were observed, which indicated that the formulation with lesser chitosan concentration would be more meaningful in terms of easy removal and application. No change in hardness and compressibility was observed at 35°C while using chitosan at 0.5% w/w indicated its non-suitability for formulation development. Chitosan at 1.5% w/w resulted in high compressibility, which might have obscured normal vision. Thus chitosan in 1.0% w/w was suggested as optimum concentration in formulation development. Increase in mucoadhesiveness was observed with increasing chitosan concentration in the formulation. Scintigraphic studies also confirmed the better retention of the developed formulation when compared with conventional eye drop formulation (**Figure 4**).

Table 4. List of *in situ* gelling system sensitive to multiple stimuli explored in ophthalmic drug delivery.

Ingredients/polymers	Drug	Strategy used	Key findings	Ref.
Poloxamer 407, Poloxamer 188, Carbopol 1342P NF	Puerarin	Temperature- and pH-induced gelation in addition to mucoadhesive property	AUC up to 5.26 times greater than drug in STF IOP-lowering effect up to 24 h than drug in STF (8 h)	[33]
Pluronic F127, Pluronic F68, Carbopol	Diclofenac sodium (DS)	Combination of temperature- and pH-induced gelation	2.2-fold higher C_{max} in comparison with conventional eye drop formulation	[35]
Chitosan, Pluronic F127	Timolol maleate	Combination of pH- and temperature-sensitive polymer	Slow release up to 10 h Higher transcorneal permeation due to penetration-enhancing effect of chitosan	[41]
Chitosan, Pluronic F127, Poloxamer 188	Ciprofloxacin hydrochloride	Combination of pH- and temperature-sensitive polymer	Newtonian flow at non-physiologic, pseudoplastic at physiologic conditions Release reported to be by Fickian or diffusion mechanism irrespective of the components	[42]
Chitosan, PF-407	_	Combination of pH- and temperature-sensitive polymer	Increased mechanical and mucoadhesive strength Fourfold increased retention compared with eye drops	[43]
Gellan gum, Chitosan	Timolol maleate	Combination of pH- and ion-activated polymer	Formulation was found to be non-irritant to mild irritant and well tolerable Formulation showed good spreading and retention for longer period	[45]
Carbopol, Poloxamer	Pilocarpine hydrochloride	Combination of pH- and temperature-induced gelation	Slower release up to 6 h 1.85 times higher mitotic response in comparison with drug in STF	[52]
Na alginate, Poloxamer	Pilocarpine hydrochloride	Combination of temperature- and ion-activated polymer	4.38-fold total mitotic response in comparison with drug in STF	[64]
Alginate	5-FU	Ion-activated gel loaded with PLA nanoparticles	Sevenfold increase in AUC compared with drug solution Higher MRT and $t_{1/2}$ compared with PLA nanoparticles and drug solution	[68]
Chitosan, Poloxamer	Fluconazole (FLU)	Combination of pH- and temperature-induced gelation	3.5-fold higher total amt. of FLU permeated when compared with aqueous drug solution	[83]
Gellan gum, Chitosan, Na alginate, Xanthan gum, Carrageenan, HPMC	Pilocarpine hydrochloride	Comparison of ion-activated *in situ* gelling system in terms of physicochemical behavior and *in vitro* drug release	HPMC and chitosan did not show any structural change upon addition of cations Gellan gum and carrageenan exhibited significant increase in hardness and viscosity upon addition of Ca^{++} and K^{+}	[84]
Gellan gum, Chitosan, Na alginate, Xanthan gum, Carrageenan, HPMC	Pilocarpine h ydrochloride	Comparison of ion-activated *in situ* gelling system in terms of precorneal retention and *in vivo* pharmacodynamics	Near about 80% of radioactivity was retained over the corneal surface in comparison with drug solution (40%) during 15 min Gellan gum, Alginate Na and carrageenan exhibited 2.5-fold AUC as compared with drug solution	[85]

In continuation, to explore the potential of chitosan-based *in situ* gel system, it was tried in combination with HPMC by our group with special emphasis on radiolabeling procedure, its optimization, *in vitro* stability of radiolabeled complex and scintigraphic evaluation of the developed system [44]. The most interesting finding of the study was a clear visible evidence of retention of the developed formulation reflected by scintigraphic images (**Figure 5A**) and time–activity curve (**Figure 5B**).

Following instillation, the plain drug formulation rapidly cleared away from the site of administration through nasolacrimal drainage and reached the systemic circulation. By contrast, the *in situ* gel was retained at the site of application even after 2 h of administration. Remarkably, no radioactivity was observed in systemic circulation for mice administered with *in situ* gel formulation. Furthermore, in quest to find the better, a unique combination of chitosan with gellan gum was also reported by our group [45]. Herein, we tried to exploit the advantage of stimulation with multiple mechanisms. Instantaneous phase transition was supposed to be due to activation of the system by three mechanisms, that is, temperature, pH and ions presented by the ocular site. This newly developed combination was comparable for various *in vitro* parameters such as physicochemical properties, transcorneal permeation and ocular irritation and proved better in terms of instantaneous gelation and better retention as indicated by time–activity profile. In a recent study, chitosan has been reported for the first time in combination with disodium glucose phosphate (DGP) for the delivery of levocetirizine dihydrochloride. Developed system exhibited sustained release and was more effective in producing antiallergic conjunctivitis effects compared with drug aqueous solution [46].

3.2.2 Synthetic polymers

3.2.2.1 Carbomers

Carbomers are the poly (acrylic acid)-based high-molecular weight polymers commercially known as Carbopol® (**Figure 2H**). These polymers exhibit sol to gel transition in aqueous solution as the pH is raised above its pKa of about 5.5. Carbopols are available in a range of molecular weights with linear, branched and cross-linked side chains. Carbopols exhibit excellent mucoadhesive property in comparison with other natural or synthetic polymers (e.g., cellulose derivatives) and hence studied extensively in ocular drug delivery. Among the carbopols, Carbopol 934 is the most commonly reported polymer composed of 62% of carboxyl groups formed by repeating units of acrylic acid, cross-linked with either allylsucrose or allylethers of pentaerythritol. Such a high bio-adhesion shown by Carbopol is possibly favored by any one of the following mechanisms *viz* electrostatic attraction (between positively charged sialic acid residue on mucus and negatively charged carboxyl groups), hydrogen bonding, hydrophobic interaction and interdiffusion. The presence of carboxyl groups on the surface of Carbopol renders its aqueous solution acidic and subsequently induces tissues irritation, when used in high concentration. In order to circumvent this problem, different combinations of Carbopol have been investigated. Carbopol, in very less concentration (0.3%), was reported in combination with MC (1.5%), which resulted in a formulation with low viscosity forming a strong gel under simulated physiological conditions [22]. Similar type of delivery system was developed by the same group using Carbopol in combination with HPMC. Results elucidated that *in situ* gel system of Carbopol in very low concentration can be developed in combination with cellulose derivatives without compromising the *in situ* gelling behavior and viscosity of the system [47]. After confirming the performance of the system in terms of its *in situ* gelling sensitivity and viscosity, the same combination was later tried by Srividya and coworkers for sustained delivery of ofloxacin. The system was developed using Carbopol 940 (0.5%) in combination with HPMC (Methocel E50LV) (1.5%) and a detailed evaluation was done by covering lots of other parameters including rheology, *in vitro* release, antimicrobial efficacy, ocular irritation and accelerated stability. The final formulation was reported to be therapeutically efficacious, stable and non-irritant while enabling sustained release up to 8 h [48]. Later, the same system but with different grades of polymers was reported for the delivery of puerarin. Carbopol 980 (0.1%) in combination with HPMC (Methocel E4M) (0.4%) and hydroxypropyl-β-cyclodextrin (HP-β-CD) (5%) was reported as optimized system for the delivery of puerarin. The most interesting finding of the work was that the polymers were used in very low concentration in comparison with previous reports without compromising the *in situ* gelling property and gelling strength of the reported system. A significant increase in puerarin permeation and low concentration of polymers used might be the effect of HP-β-CD used in 5% w/v concentration [49]. We reasoned that Carbopol, being a poly acrylic acid derivative, might show incompatibility with certain drugs. Keeping this point in mind, sustained release *in situ* gel composed of Carbopol 940NF and a different grade of HPMC (Methocel® K100LV) was developed for once-a-day ocular delivery of ciprofloxacin hydrochloride. Ciprofloxacin hydrochloride was complexed with ion exchange resin, Indion® 254F (chemically polystyrene cross-linked with divinylbenzene) before adding to the final formulation for avoiding incompatibility [50]. In a recent study, *in situ* gel system has been also reported for the poorly water-soluble drug, dexamethasone (DXN). To improve the solubility and permeability of DXN, the drug was complexed with HP-β-CD and incorporated in pH-induced mucoadhesive hydrogel composed of Carbopol 980NF (0.2% w/v) in combination with sodium carboxymethyl-cellulose (Na CMC) (0.4% w/v) [51]. This finding supported the previous finding by Wu *et al.*, 2007, in which HP-β-CD was reported to increase stability and permeability, and reduced concentration of gelling polymer was needed for the fabrication of formulation [49].

Although ample numbers of reports are available for Carbopol in combination with other bio-adhesive cellulose derivatives, another combination with Poloxamer has also been investigated extensively. Carbopol in combination with Poloxamer was studied by Lin and Sung for the delivery of

pilocarpine hydrochloride and 0.3% w/v Carbopol with Poloxamer 14% w/v was reported as optimum concentration for *in situ* gel formation with significant gel strength under physiological conditions. The slower release up to 6 h and significant higher mitotic response, 1.85 times by Carbopol/Poloxamer combination, were observed in comparison with drug in simulate tear fluid (STF) while it was 1.24 and 1.5 times by Carbopol and Poloxamer alone [52]. The finding clearly reveals the additive effect of combination in comparison with polymers used alone. **Table 2** summarizes pH-sensitive *in situ* gelling systems that have been explored for ophthalmic applications till date.

3.3 Ion-sensitive gelling system

Certain polymers undergo phase transition in presence of ionic environment, which is provided by ocular site (Ca^{++} and other ions present in tear fluid). Hence, this property has been studied extensively for developing the *in situ* gel system for ocular delivery. For the purpose, gellan gum, alginates and β-Carrageenan have been widely investigated.

3.3.1 Gellan gum

Gellan gum is a linear, anionic heteropolysaccharide comprising glucose, glucuronic acid and rhamnose in the molar ratio 2:1:1 as polymer backbone linked together to give a tetrasaccharide repeat unit (**Figure 2I**).

This is microbial in origin and secreted by *Sphingomonas elodea*. Commercially it is known as Gelrite®, which is a deacylated product of the naturally occurring polysaccharide. Although it is of microbial origin, it is safe and efficacious, which is reflected by its regulatory approval as pharmaceutical excipient and controlled-release commercial product (Timoptic XE) for glaucoma treatment. The aqueous solution of gellan gum is a low-viscosity solution, which shows very good flowability and is converted into a stiff gel because of cross-linking of the negatively charged polysaccharide helices by monovalent and divalent cations (Na^+, K^+, Ca^{++}) present in tear fluid. Double helices formation and weak association between them in an ion-free environment lead to solution with low viscosity while cation-mediated aggregation and helices association in presence of cations result in gel formation [53]. Divalent cations, magnesium (Mg^{++}) and calcium (Ca^{++}), have been reported to be superior in causing gelation in comparison with monovalent cations [54]. However, the higher concentration of sodium in tear fluid (2.6 g/l) is also sufficient to induce gelation. Ample number of reports is available in which gellan gum has been used as delivery vehicle. Herein, the discussion will be restricted to some of the interesting findings. In early studies, Gelrite (0.6%)-based *in situ* gel containing timolol maleate was developed and compared with hydroxyl ethyl cellulose (HEC, 0.5%) *in situ* gel [55]. The performance of the system developed using Gelrite was greater in terms of timolol concentration in aqueous humor, cornea and iris + ciliary body. In later reports, the system was evaluated for sol–gel transition by dynamic and static light scattering techniques [56], functionality testing [57] and rheological evaluation under physiological conditions [58,59]. Balasubramaniam *et al.* studied this system for the delivery of indomethacin [60] and ciprofloxacin hydrochloride [61]. Although gellan was used in concentration of 0.6% in some previous reports, it caused gelation upon cooling to 40°C beyond the concentration of 0.5% as an important finding of their study. This system was further taken into consideration by Sultana and coworker for the delivery of perfloxacin mesylate. The developed formulation was highly stable (> 2 years), sterile, passed the test for antimicrobial efficacy and could effectively sustain the release up to 12 h [62]. To further measure the synergistic effect, a combinatorial approach was taken into account by our group and a unique combination of gellan (ion-activated gelation) with chitosan (pH-activated gelation) was explored for the delivery of timolol maleate, the drug used frequently for glaucoma therapy. The developed formulation was clear, gelled at physiological pH with sufficient high viscosity to retain at corneal surface. Formulation was non-irritant and exhibited better performance in terms of *in vitro* transcorneal permeation when compared with simple gellan gum formulation and free drug solution. The observed difference might be attributed to penetration-enhancing effect of chitosan. Gamma scintigraphic study confirmed the better retention in comparison with plain drug solution [45].

3.3.2 Alginates

Alginate is a linear co-polysaccharide composed of (1→4) linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues (**Figure 2J**). Characteristic properties of alginate hydrogels, such as mechanical strength and porosity of the gel, are highly dependent on the G:M ratios, type of ionic cross-linker, concentration and viscosity of the solution. Preferential interaction of calcium ions with the G moieties is supposed to be responsible for three-dimensional gel formation. Alginate with a high guluronic acid content (more than 65%) has been reported for instantaneous gel formation and slow release up to 24 h in comparison with alginate with low guluronic acid content. Alginate *in situ* gel loaded with pilocarpine as model drug and composed of high guluronic acid content has been reported to reduce IOP up to 10 h while IOP lowering was up to 3 h for drug solution [63]. In order to explore its efficacy in combination, alginate (2%) was combined with Poloxamer (14%) and reported to perform better in comparison with alginate and Poloxamer alone [64]. Alginate has been also tried in other combinations with viscosity-enhancing agents (HPMC). In majority of the cases, the combination is reported to perform better in comparison with alginate/HPMC alone [65]. **Table 3** summarizes ion-activated *in situ* gelling systems that have been explored for ophthalmic applications till date.

4. Current trends in *in situ* gel research

A scrupulous look at research done in last decade clearly reveals the inclination of the researchers toward the combination approach. Initially, a number of natural and synthetic

polymers were identified, synthesized and used for *in situ* gel formation but later, considering the synergistic effects of polymeric combinations, these systems were taken into consideration and successfully utilized for the fabrication of engineered ocular delivery systems with optimized efficacy. Identification of suitability and efficacy of combination approach persuaded their further exploration in terms of ocular deliverability. Use of different combination not only reduces the concentration required of individual polymer but also fortifies the response in terms of making system responsive to multiple stimulations. The combination approach also augmented the therapeutic effectiveness of the drug entrapped inside the polymeric matrices. In a recent study by Ammar *et al.*, *in situ* gel nanoemulsion containing dorzolamide hydrochloride was reported as an alternative to conventional eye drops. The formulation was even better than conventional eye drops in terms of its efficacy as well as simplicity of fabrication [66]. The interesting part of the work was to take advantage of dual properties of Poloxamer, that is, surface-active properties and thermosensitive sol–gel phase transition. The formulation was optimized for concentration of its components by considering the effect of various parameters *viz* droplet sizes, gelation temperature, *in vitro* release and biological response (IOP-lowering effect) and so on. The developed formulation showed faster onset of action (in decreasing IOP) in comparison with both drug solution and marketed product. Interestingly, its action was continued up to 8 h in contrast to 3 and 4 h for drug solution and marketed product, respectively. The AUC value of the *in situ* gel nanoemulsion was four times higher than that of the drug solution and at least twice than the marketed product. The developed *in situ* gel nanoemulsion was also superior in terms of its biological response when compared with plain *in situ* gel formulation, deprived of the oil present in the nano-emulsion [66]. In a very recent study, a multicomponent *in situ* gel system is reported for the delivery of moxifloxacin hydrochloride. The novelty of the system lies in selection of components in system fabrication. The system comprises of polyox (a pH-sensitive gelling agent), sodium alginate (an ion-sensitive gelling agent) and Poloxamer (a temperature-sensitive gelling agent) along with HPMC K4M as viscosifying agent. The system is supposed to be responsive against multiple mechanisms with improved performance in terms of superior retention. The system was therapeutically effective, stable, non-irritant and able to sustain the release over 8 h [67]. In sequence, a hybrid-modified nano *in situ* gel system consisted of poly lactic acid (PLA) nanoparticles dispersed in sodium alginate solution for the delivery of 5-fluorouracil (5-FU) was reported [68]. The AUC of the presented system was seven- and twofold higher as compared with drug solution and PLA nanoparticles, respectively. Current trends clearly indicate that this field is now turning toward the combination or hybrid approach to take the advantage of multiple polymers/techniques. **Table 4** summarizes *in situ* gelling systems sensitive to multiple stimuli and hybrid systems that have been explored for ophthalmic applications till date.

5. Expert opinion

Over the past two decades, researchers have significantly strived to work out a variety of novel approaches that can prolifically obviate the obstacles endowed with conventional ocular formulations. Development of *in situ* gel-based delivery systems is undoubtedly one of the best fruits of these exercises. Ever since their introduction in the pharmaceutical regimen, the field of ocular delivery has marched with exceptional tempo; the enthusiasm has been reflected on ample number of published reports and ongoing researches in the relevant area.

The most critical challenge in the field of ocular delivery is to prolong the contact time of the administered formulation with the eye tissues and finally improve their ocular BA. Excitements encompassing *in situ* gel systems may be ascribed to their unique phase transition properties (sol–gel transformation), which not only facilitate their administration as drop form but also create fewer problems associated with vision. Moreover, these systems offer good sustained-release properties and drop off the systemic absorption and deleterious effects of the drug entrapped by the polymeric matrices. As elaborated in the current review, it has been possible to develop an array of polymers, which, alone or in state of combination, undergo *in situ* gelation with response to changes in external stimuli. Needless to describe, this stimuli-responsiveness form the basis of system engineering discussed so far and has been manifested through changes in physical properties of the instilled formulation. Thus, we can see that *in situ* gel systems are comparable with conventional eye drops in terms of their ease of administration in drop form. Remarkably, as for efficacy is concerned, these formulations are far more superior to normal eye drops in terms of their prolonged retention time or contact with the eye tissues and enhanced ocular BA. It will be further interesting to note that in the early era of their introduction, *in situ* gel systems were fabricated using polymers that were supposed to show phase transition by only one mechanism, that is, either temperature or pH or ionic strength of the bio-environment. Gradually, these systems have been tried in diverse combinations with an aim to improve their responsiveness toward multiple stimuli. These combination systems prompt the development of 'smart' ocular delivery platforms, which, depending on specific physiological requirements, proffer an assortment of important properties and functions that, usually, are not available with the single or isolated polymer.

As for the clinical status is concerned, Timoptic XE, timolol maleate containing gellan gum-based formulation and Rysmon® TG timolol maleate (Hanmi Pharmaceutical Co., Ltd., Tokyo, Japan) containing MC, sodium citrate and polyethylene glycol-based formulation are the *in situ* gel products currently available in the market. Although lots of research has been done in the area, still more in-depth and rigorous studies are required so that replacement of conventional eye drops

with *in situ* gelling systems comes into fruition, some day. The major challenges still rest in

- complexity of the system in terms of multiple components,
- their responsiveness toward the bio-stimulus,
- regulatory issues pertinent to toxicity of the components used,
- reproducibility in terms of its performance,
- industrial applicability and
- stability and sterility of the formulation developed.

Besides complexity of preparation, higher cost of these formulations (as compared with simple eye drops) coupled with regulatory issues (pertinent to toxicity of the polymers forming the gel) averts their industrial scale-up and subsequent translation to clinics. This might be a major factor why only few *in situ* gel-based formulations are present in the market. Researcher should focus on these hurdles and work out how the cost of the formulations can be reduced without compromising with their performance. This goal can be partially realized by reducing the complexity of the system and substituting the components with cheaper alternatives with comparable efficacy. In the current scientific panorama, combination and hybrid formulation approach have opened a new transom, which should be further explored in order to develop 'smarter' ophthalmic delivery systems. However, special care should be taken to address the toxicity issues pertaining to their constituents so as to avoid any regulatory hurdles during commercialization. The synthetic polymers that are commonly used for the development of *in situ* gels may be good enough in terms of their capability to demonstrate multiple functions and/or responsiveness toward multiple physiological stimuli. Nevertheless, their non-biodegradable nature and concentration-dependent toxicity have to be considered with equal importance and explored in details. In our opinion, it is better to switch toward natural polymers that are not only non-toxic but biodegradable too. However, in this case also, their precise production with reproducible properties has to be ensured.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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