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*Teaser This article discusses advances in the use of poloxamers as **in situ** gels for ocular drug delivery, highlighting challenges, and recommending further possible applications.*

Poloxamer-based *in situ* gelling thermoresponsive systems for ocular drug delivery applications

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In situ **gels have recently received interest as ocular drug delivery vehicles because they combine the merits of easy instillation and sustained drug release. In this review, we focus on the use of poloxamers as *in situ* gelling systems in ocular drug delivery because of their thermoresponsive gelling behaviour, biocompatibility, and ease of sterilisation. Furthermore, the sol–gel transition temperature, mucoadhesive properties, and drug release profiles of poloxamer-based *in situ* gels can be finely tuned, enabling them to be used as vehicles for the delivery of small and large drug molecules to treat diseases of the anterior and posterior segments of the eye. Poloxamer-based ocular products have already found their way to the pharmaceutical market, but remain a potential arena for further investigation and commercial exploitation.**

Introduction

The human eye can be divided into three segments; precorneal area, and anterior and posterior segments [1]. The most common route of drug administration to the eye is topical instillation of eye drops. However, the corneal uptake of drug from topically applied ocular formulations is low (i.e., <10%) [2,3]. This poor ocular bioavailability is attributed to several physiological factors, including limited permeability of the corneal membranes. Furthermore, eye drops have a short precorneal residence time of 1–2 min because of their drainage through the nasolacrimal route and/or their systemic absorption via the highly vascular conjunctiva [2,3]. This rapid precorneal elimination of eye drops leads to their frequent dosage regimen, resulting in poor patient compliance, adherence, and adverse effects. Therefore, the substitution of conventional eye drops with mucoadhesive hydrogel-based formulations can act as an effective strategy to enhance drug retention and bioavailability.

Hydrogels are hydrophilic molecules comprising a 3D network that can absorb and retain a large amount of water, making them important materials for drug delivery [4]. They can be used as vehicles to deliver drugs to the eye to prolong precorneal retention time and, in turn, improve ocular bioavailability. *In situ* gelling systems are solutions that undergo transition into semisolid gels in response to physiological stimuli, such as body temperature, physiological pH, and ionic strength of

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the biological fluids [5,6]. Thus, instillation of *in situ* gelling systems in the eye combines the merits of accurate dosing and easy administration of eye drops with prolonged retention in the eye and sustained drug delivery [5]. Furthermore, *in situ* gelling systems can act as promising vehicles for intraocular and periocular injections, where they can create depots after injection into the vitreous humour or in periocular tissues to provide sustained drug release to the posterior segment of the eye [7].

Thermoresponsive gelling systems are polymeric solutions that undergo sol–gel transitions in response to temperature changes. The lower critical solution temperature (LCST) is the minimum sol–gel transition temperature ($T_{sol–gel}$) of the polymer on its temperature-concentration phase diagram, and depends on the interactions between water molecules and different hydrophilic/hydrophobic segments in the polymeric chain [6,8]. Several natural and synthetic polymers exhibit thermoresponsive gelling behaviour at temperatures close to body temperature; thus, they can be used as injectable solutions or eye drops to achieve sustained drug delivery. For example, aqueous solutions of methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) exhibit an initial drop in viscosity upon heating, followed by solidification into hydrogels on continuous heating [9,10]. Chitosan-based thermoresponsive hydrogels were introduced by Chenite *et al*. as biocompatible sustained drug delivery systems [11] that resulted in the sustained delivery of latanoprost and ferulic acid to rabbit eyes [12,13]. Miyazaki *et al*. showed that an enzyme-degraded xyloglucan solution loaded with pilocarpine hydrochloride exhibited thermoresponsive gelling behaviour in rabbit eyes and increased the duration of the miosis [14]. Poly(*N*-isopropylacrylamide) (pNIPAAm) is a thermoresponsive polymer with a LCST of ~32 °C, which can be tuned by grafting hydrophilic monomers [15]. For instance, Derwent *et al*. and Egbu *et al*. exploited pNIPAAm crosslinked with poly(ethylene glycol) diacrylate (PEGDA) with or without hyaluronic acid (HA) for the delivery of proteins to the posterior segment of eye, where the hydrogels displayed LCSTs ranging from 31 °C to 36 °C, depending on the PEGDA content [16,17]. Gao *et al*. synthesised a poly-(DL-lactic acid-*co*-glycolic acid)–PEG (PLGA-PEG) copolymer, which exhibited a $T_{sol–gel}$ of 32 °C and was used as an *in situ* gel for the ocular delivery of dexamethasone acetate [18].

Poloxamers are synthetic polymers that exhibit thermoresponsive behaviour, with a finely tunable $T_{sol–gel}$; thus, they are used for several pharmaceutical and biomedical applications [2,19,20]. They are commercially available as Pluronics®, Kolliphors®, and Lutrols® [21]. Poloxamer 407 (P407) and poloxamer 188 (P188) are among the most commonly used poloxamers in ocular drug delivery as a result of their good solubility in water, clarity of their aqueous solutions, concentration-dependent viscosity, shear-thinning behaviour of their aqueous solutions, and their safety to the ocular tissues. In this review, we are primarily concerned with the physicochemical properties of poloxamers, particularly their thermoresponsive behaviour, and their potential applications in ocular drug delivery for both the anterior and posterior segments of the eye.

Physicochemical properties of poloxamer-based *in situ* ocular gels

Chemical structure of poloxamers

Poloxamers are non-ionic surfactants with a triblock copolymer structure comprising two hydrophilic poly(ethylene oxide) (PEO) blocks with a hydrophobic poly(propylene oxide) (PPO) block (Fig. 1a) [2,19]. Their different PEO:PPO proportions contribute to their variable physicochemical properties. Poloxamers have a unique nomenclature system comprising three digits, where the first two digits represent the approximate molecular weight (Mwt) of the PPO block divided by 100, whereas the third digit represents the approximate weight percentage of the PEO divided by 10. By contrast, Pluronics® are nominated by a letter representing their physical state followed by a three-digit number that depends on the PEO:PPO weight fraction. Pluronics® are given a letter (L) for liquid, (P) for paste, and (F) for flakes. The first two digits represent the approximate Mwt of the PPO block divided by 300, whereas the third digit represents the approximate weight percentage of the PEO divided by 10 [21]. Table 1 presents examples of different poloxamers and their corresponding commercially available Pluronics® as per the manufacturer's descriptions.

Thermoresponsive behaviour of poloxamers

Given their surfactant properties, poloxamer molecules self-associate, forming micelles at a certain concentration known as the critical micelle concentration (CMC). During micelle formation, the PPO groups interact together via van der Waals forces to form the hydrophobic micelles core, whereas the PEO groups occupy the micelle shell, interacting with water molecules via hydrogen bonds [3]. Temperature increases favour interactions between PPO groups as well as polymer desolvation, thus enhancing micelle formation at lower polymer concentrations [3]. Upon further heating of the micellar aqueous solution, poloxamer micelles aggregate at a certain temperature and the system fluidity decreases abruptly, leading to gel formation. This process is reversible because cooling converts the gel back to its original sol state (Fig. 1b) [22]. Over the past three decades, the thermoresponsive behaviour of poloxamers has been thoroughly investigated with respect to the development of sensitive and precise techniques for the determination of $T_{sol–gel}$, as well as the investigation of different molecular and formulation variables affecting their thermoresponsive gelling behaviour.

Measurement of $T_{sol–gel}$ of poloxamers solutions

Different techniques have been developed for the determination of $T_{sol–gel}$. The simplest method is test tube inversion, where a test tube containing the sample is repeatedly tilted in a gradually heated water bath and the temperature at which no flow occurs is then recorded (Fig. 2a) [23]. Another simple method includes heating the sample gradually on a magnetic stirrer and recording the temperature at which the magnetic bar stops moving [24]. Both of these visual observation methods offer rapid determination of approximate $T_{sol–gel}$ with minimal equipment, yet, their results are not reliable enough in terms of accuracy and precision [3].

Other methods for determining $T_{sol–gel}$ include scanning the ultraviolet (UV)-visible absorbance of polymeric solutions at 500 nm with a gradual increase in temperature, where an absorbance peak is observed at the gelation point [25]. Similarly, dynamic light scattering (DLS) can trace the aggregation of micelles by measuring the hydrodynamic diameters in polymeric solutions at different temperatures and concentrations [26,27]. Furthermore, the gelation process is associated with a secondary endothermic peak, which can be detected using microdifferential

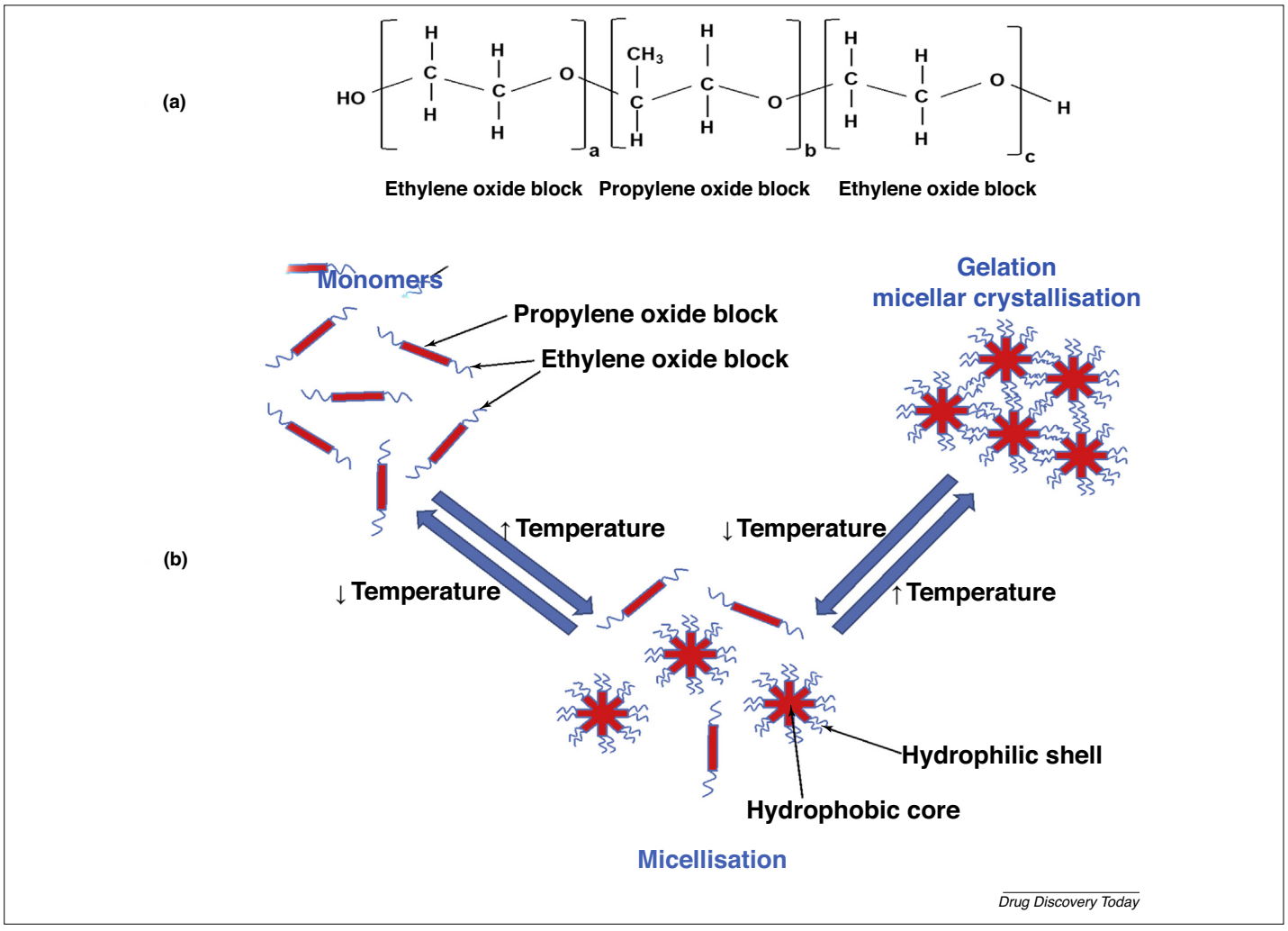

FIGURE 1

Schematic of (a) the chemical structure of poloxamers and (b) molecular phase changes exhibited when the temperature is changed.

TABLE 1

Commonly marketed poloxamers

Poloxamer	n^a	m^b	Molecular weight (g/mol)	Commercial name (Pluronic®)
124	12	20	2090–2360	L44 NF
188	80	27	7680–9510	F68 NF
407	101	56	9840–14 600	F127 NF
237	64	37	6840–8830	F87 NF
338	141	44	12 700–17 400	F108 NF
235	26	40	4600	P85

[a] *n*, number of units of ethylene oxide on each side of the polymer molecule.
[b] *m*, number of units of propylene oxide in the polymer molecule.

scanning calorimetry (micro-DSC) (Fig. 2b) [28]. UV spectroscopy, DLS, and micro-DSC yield accurate results of the $T_{sol–gel}$, but do not give insight into changes in the rheological behaviour at $T_{sol–gel}$.

Rheological measurements can be done using temperature-controlled rotational viscometers, where the sample is heated slowly at a constant shear rate and $T_{sol–gel}$ is determined as the temperature at which the sample exhibits an abrupt increase in viscosity [3]. However, the rate of temperature increase has a crucial effect on the accuracy of this method, where rapid temperature increases can result in a higher false $T_{sol–gel}$ being recorded, because of the time elapsed during gel-network formation and the subsequent increase in sample viscosity. $T_{sol–gel}$ can be determined more accurately by operating the rotational viscometer in oscillation mode, where the sample temperature is increased slowly with concurrent measurement of both the elastic (storage, G′) and the viscosity (loss, G″) moduli. The elastic modulus is proportional to the energy stored and returned on oscillation, whereas the viscosity modulus is proportional to the energy dissipated in friction. Therefore, G′>G″ in predominantly elastic solids, whereas G″ > G′ in predominantly viscous liquids.

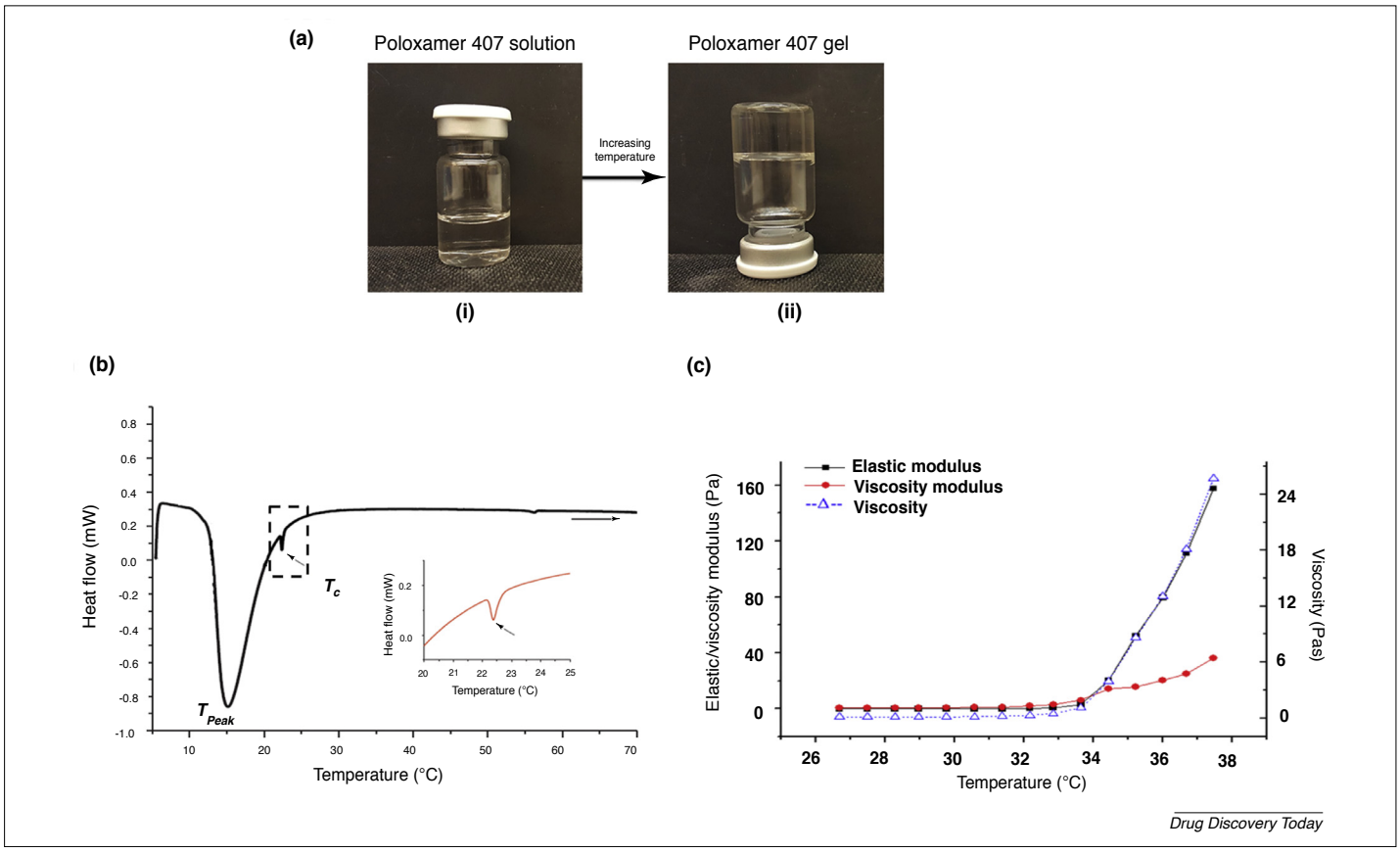

FIGURE 2

Methods for determining the sol–gel transition temperature. (a) Digital image of poloxamer formulation in sol form (i), and in gel form (ii). (b) DSC thermogram of poloxamer 407 (20% w/w) showing the sol–gel transition at 24 °C. (c) Viscosity, storage, and loss moduli of poloxamer formulation showing sol–gel transition at 34 °C. Reproduced with permission from [20] (a) and [28] (b), and from [29] under CC BY license (c).

$T_{sol–gel}$ can be determined as the point at which G′ and G″ intersect **(**Fig. 2**c)** [27,29,30].

Optimisation of $T_{sol–gel}$ *of poloxamer solutions for ocular use*

The optimum *in situ* gelling formulation should have a $T_{sol–gel}$ above room temperature and below the precorneal temperature (25–34.5 °C), ideally 30 ± 2 °C [31]. However, P407 aqueous solutions exhibit unsatisfactory $T_{sol–gel}$ values below room temperature at concentrations 20–30% w/w, whereas P188 aqueous solutions have $T_{sol–gel}$ >40 °C at the same concentrations. Therefore, mixtures of both poloxamers are commonly used in different proportions to yield desired $T_{sol–gel}$ [32]. Increasing the proportion of P407 decreases the $T_{sol–gel}$, because the increased number of micelles decreases the energy needed for endothermic micellar crystallisation, resulting in subsequent gelation at lower temperature [28]. By contrast, increasing the proportion of the more hydrophilic P188 disrupts P407 micelles, which results in increasing the energy required by P407 to undergo hydrophobic interactions, with a subsequent increase in $T_{sol–gel}$ [32,33]. Nevertheless, the effect of P188 is reversed at concentrations >10% w/w, where its molecules participate in constructing the gel network, which decreases $T_{sol–gel}$ [34,35]. At these concentrations, the gel formation can be explained by the jamming effect of micelles rather than by micellar crystallisation [28]. Formulations containing P188 at concentrations ≥20% w/w have poor gelling properties [24]. Thakur *et al.* reported that poloxamer 237 affected the $T_{sol–gel}$ of P407 solutions in a way similar to P188 [36].

In 2002, Wei *et al.* used multivariable regression analysis to propose Eq. 1 for calculating the $T_{sol–gel}$ of P407/P188 mixtures [34]:

$$T_{sol–gel} = 87.13 – 2.65\ C_{407} – 0.41\ C_{188}, \quad (1)$$

where C_{407} and C_{188} are w/w percentages of P407 and P188, respectively.

In 2010, Qian *et al.* modified the previous equation to form Eq. 2 [35]:

$$T_{sol–gel} = 277.08\ C^2_{407} – 737.03\ C^2_{188} – 409.03\ C_{407} + 1.07\ C_{188} + 566.09\ C_{407}\ C_{188} + 97.78. \quad (2)$$

Before making optimum poloxamer preparation, the effect of other formulation components on $T_{sol–gel}$ must be taken into consideration. Drug addition will influence the $T_{sol–gel}$ of the poloxamer formulation; for example, Kim *et al.* reported that incorporation of 0.5% w/v recombinant human endothelial growth factor (rhEGF) complex with hydroxypropyl-β-cyclodextrin (HP-β-CD) into a P407/P188 solution increased $T_{sol–gel}$ by 1 °C [37]. Furthermore, addition of Tween 80 at concentrations 0.5–1.5% w/w increased the $T_{sol–gel}$ of 20% w/w P407 solutions by 6–11 °C [38]. By contrast, Krtalić *et al.* showed that low Mwt drugs decreased the $T_{sol–gel}$ of P407/P188/chitosan solutions in order of their hydrophobicity [31]. Similarly, both El-Kamel and Qian *et al.* demonstrated that the addition of isotonicity modifiers, such as sodium chloride, mannitol, and sorbitol, to poloxamer solutions decreased $T_{sol–gel}$ by 2–3 °C [35,39].

Alexandridis and Holzwarth also studied the effect of different salts on $T_{sol–gel}$ and explained this effect through the salting-out phenomenon, which is correlated with the ionic radius of the added salts and their solvation heat [40].

The ionic strength of tear fluids and their dilution effect on poloxamer solutions should also be considered [34,41]. Jiang *et al.* reported that $T_{sol–gel}$ of P407/P188 mixtures was higher in simulated tear fluid (STF) relative to purified water, although the study relied solely on visual observation of a rotating magnetic bar to determine $T_{sol–gel}$ [41]. By contrast, Al Khateb *et al.* used a more accurate micro-DSC method to show that the salt composition of the STF did not have a significant effect on the micelle formation of P407/P188 mixtures relative to purified water [26]. Furthermore, researchers should pay particular attention to poloxamer purity; for example, Fakhari *et al.* adopted a solvent-extraction purification process for compendial P407 and showed that the solutions of the purified P407 had lower $T_{sol–gel}$ than the corresponding solutions of unpurified P407 [20].

Mechanical and rheological properties of poloxamer-based gels

The mechanical and rheological properties of poloxamer-based gels are closely correlated and directly affect the gel retention time within the eye, where the relatively weak mechanical strength and low viscosity of poloxamer gels can contribute to their relatively rapid erosion [42]. Nonetheless, high mechanical strength and viscosity are generally unfavourable because of the difficulty of application and patient inconvenience [43]. Therefore, optimum mechanical strength and viscosity provide a balance between easy administration and long retention time within the eye [24]. The mechanical properties of poloxamer-based gels can be assessed using a TA-XT texture analyser at physiological temperature, where the gel hardness is measured as the force of gel compression, expressing the applicability of the gel to the eye, whereas the gel compressibility is measured as the work required to deform the gel, expressing its spreadability on the ocular tissues [24,43,44]. By contrast, gel viscosity at physiological temperature can be measured using rotational viscometers.

Baloglu *et al.* showed that poloxamer gel hardness, compressibility, and viscosity increase with increasing total poloxamer concentration [24]. Additionally, formulations with higher P407:P188 ratios show higher hardness, compressibility, and viscosity. Nevertheless, other polymers can be added to improve the mechanical and rheological properties of poloxamers gels. For instance, Gratieri *et al.* and Pandey *et al.* increased the hardness, compressibility, and viscosity of P407 gels by adding either chitosan or HPMC/chitosan mixtures at concentrations 0.4–1.5% w/w [30,45]. In two separate studies, Ferreira *et al.* enhanced the hardness, compressibility, and viscosity of P407 gels by adding either Carbopol® 974 or polycarbophil at concentrations 0.1–0.5% w/w [43,44]. However, the addition of other polymers to poloxamers solutions can affect the $T_{sol–gel}$. Therefore, researchers should pay attention to the effect of the formulation additives on all of the properties of the gel formulation.

Mucoadhesive properties of poloxamer-based gels

Mucoadhesion is an important property of ocular gels to enhance their retention within the eye, prolong drug release, and minimise formulation clearance. Choi *et al.* developed an *in vitro* method to measure the mucoadhesive force of poloxamer liquid suppositories on the rectal membrane, by measuring the force required to detach two pieces of the rectal membrane with poloxamer gel in-between them [46]. The force was applied by adding weights to one side of a lever, while the other side was tied to the upper piece of membrane. This method was adapted by Shastri *et al.* to test the mucoadhesiveness of poloxamer gels to corneal membrane by replacing the rectal membrane with excised sheep cornea (Fig. 3) [47]. Qi *et al.* modified this device by replacing the weighing pan with a dripping infusion set to fine tune the detachment force [48]. The lower membrane was also replaced with a thermostatically controlled platform for

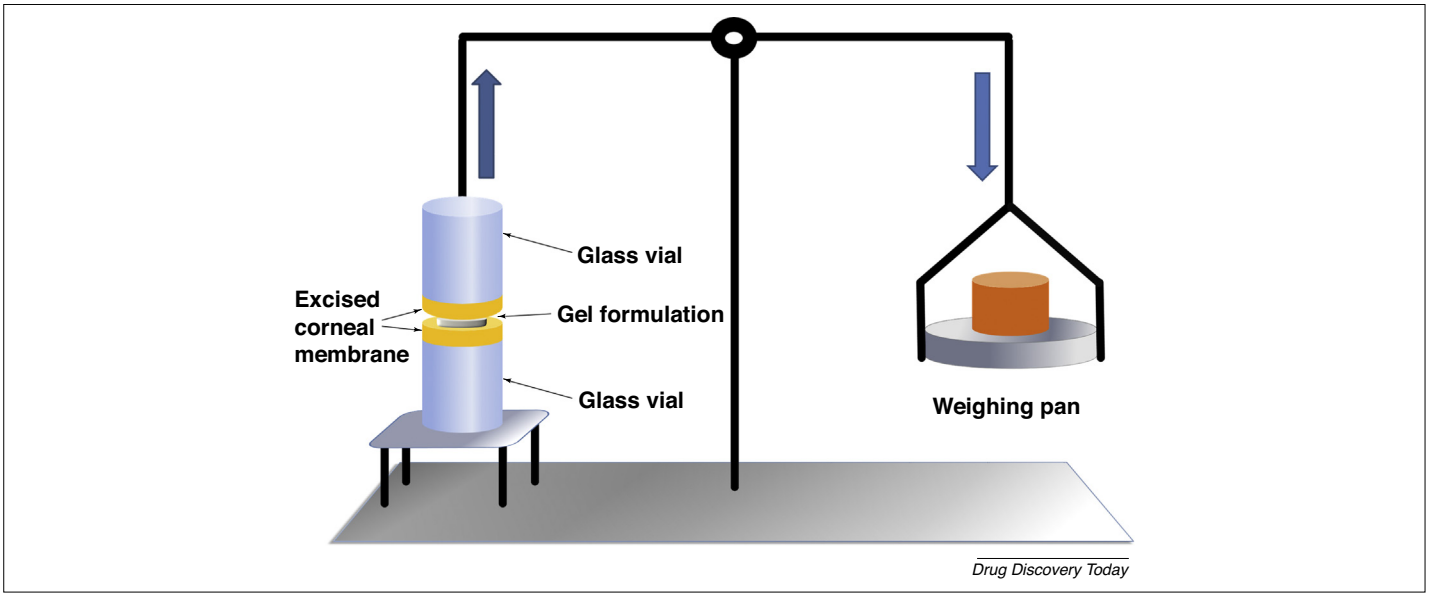

FIGURE 3

Device for determining the mucoadhesive force of a gel formulation against the corneal membrane according to [47].

maintenance of the gel at physiological temperatures. Mucoadhesive force can also be measured *in vitro* by using a TA-XT texture analyser, where mucin discs are attached to the probe using double-sided adhesive tape and brought into contact with the gel formulation, followed by measurement of the force required to detach the mucin disc from the gel [30,33]. Similarly, the mucoadhesive force of the gel against the corneal tissues was measured using the peel test method, where poloxamer gels are pressed against excised rabbit cornea using an Instron® testing machine, followed by raising the probe to measure the detachment force between the gel and the corneal tissues [37]. Mucoadhesive force can be also determined by evaluating the rheological synergism between mucin and polymer formulation, where viscosities of mucin (η_m), polymer formulation (η_p), and mixture of both (η_t) are measured at a certain shear rate, and used to calculate the viscosity component of mucoadhesion (η_b) using Eq. 3 [26,27]:

$$\eta_b = \eta_t - (\eta_m + \eta_p). \quad (3)$$

The mucoadhesive force (F_b) can be calculated using Eq. 4 [26,27]:

$$F_b = \eta_b . \gamma\}' \backslash hbox \{. \quad (4)$$

where γ' is the shear rate at which viscosity is measured.

P407/P188 mixtures have certain mucoadhesive properties. For example, Fathalla *et al.* showed that the work of adhesion increased when the concentration of P188 was increased up to 15% w/v, whereas it decreased on increasing P407 above 23% w/v [33]. Other polymers can be added to improve mucoadhesiveness, such as chitosan (1% w/w), which increased the mucoadhesive force of 18% w/w P407 gels by 26% [30]. Addition of 0.1% w/v Carbopol® 1342 resulted in a threefold increase in the mucoadhesive force of a poloxamer mixture comprising of 21% w/v P407 and 5% w/v of P188 [48]. Similarly, Cao *et al.* showed that the addition of 0.3% w/v Carbopol® 974 resulted in 2.3-fold improvement in the mucoadhesive force, which was associated with a slight decrease in $T_{sol-gel}$ [49]. Gelrite and low Mwt HA (150 kDa) were also shown to improve the mucoadhesive force of P407 gels [27,47]. This was associated with an increase in the gel strength and a subsequent decrease in $T_{sol-gel}$. By contrast, the addition of 0.2% w/w high Mwt sodium hyaluronate (1200 kDa) to the poloxamer formulation hindered micelle formation, decreasing the gel strength; hence, the precorneal retention time was not improved [34]. Therefore, the probable adverse effects of added polymers on the mechanical, rheological, and thermoresponsive properties of the formulation must be thoroughly investigated.

Ocular applications of poloxamer-based *in situ* gels

Historical overview

As early as 1982, Miller and Donovan published a study investigating the miosis obtained in rabbit eyes after application of pilocarpine nitrate incorporated in a 25% w/v P407 gel [50]. The authors reported that the gel formulation exhibited a 1.9-fold increase in response of miosis relative to a corresponding aqueous pilocarpine nitrate solution, although the gel formulation was diluted with tear fluid and washed out within 5 min. In 1987, Gurny *et al.* reported the tracing of the precorneal clearance of pilocarpine hydrochloride gel formulations from rabbit eyes using gamma scintigraphy, where ~80% of the 25% w/w P407 gel formulation was washed out from the corneal surface within 10 min, which was approximately four times slower than the precorneal clearance of a pilocarpine hydrochloride aqueous solution [51]. However, one should be cautious about considering the formulations prepared in these studies as *in situ* forming gels, because P407 solutions underwent a sol–gel transition at room temperature before application to the rabbit eyes. Nevertheless, these preliminary studies opened the door for further investigation of the possible applications of poloxamers as *in situ* thermoresponsive gel-forming polymers for ocular preparations.

During the late 1980s, Saettone *et al.* investigated the use of different poloxamers as solubilisers for tropicamide [52]. The authors described the gel-forming properties of poloxamers on instillation in the eye and excluded the *in situ* gelling formulation as a result of what was considered to be its undesirable rheological behaviour. In another study published in 1989, Saettone *et al.* exploited the solubilising and *in situ* thermoresponsive gelling properties of poloxamers in the development of forskolin solutions for ocular use, where the forskolin gelling solution exhibited prolonged intraocular pressure (IOP)-lowering activity in a rabbit model relative to a forskolin suspension [53]. The improvement in solubility of both tropicamide and forskolin did not result in subsequent improvement of ocular bioavailability because of binding of the drugs inside the poloxamers micelle.

Effect of sterilisation

The major challenge facing the formulation of ocular gels is their sterilisation. Most polymers cannot withstand common sterilisation techniques, where the application of heat, radiation, or chemical sterilisation can trigger reactions leading to loss of the polymer gelling properties. Different studies have investigated the stability of poloxamers gels in response to steam sterilisation, where *in situ* gels comprising mixtures of P407, P188, Tween 80, chitosan, and carbopol were deemed stable against autoclave sterilisation at 121 °C and 15 psi for 20 min in terms of their $T_{sol-gel}$ and flow behaviour [32,38,54]. By contrast, thermosensitive drugs can be sterilised by membrane filtration of their solutions followed by crystallisation and incorporation into the steam-sterilised polymer formulation under aseptic conditions [55,56].

Preservatives are added to multidose ophthalmic preparations to maintain their microbiological qualities during use. However, care must be taken concerning the influence of preservatives on the flow behaviour of the *in situ* gelling systems. A recent study by Boonlai *et al.* showed that P407 solutions of concentrations 16–20% w/w exhibited a 2 °C decrease in their $T_{sol-gel}$ upon addition of 0.2% w/w methylparaben, which could be attributed to the ability of methylparaben to promote polymeric gelation through the association between micelles [57].

Ocular biocompatibility of poloxamer-based gels

The safety of poloxamers for topical ocular use has been thoroughly investigated in several studies using different techniques. Mucous production by the slug *Limax flavus* was used as a preliminary test of the biocompatibility of poloxamers, and showed their general safety on mucosal tissues [26].

Several *in vitro*, *ex vivo*, and *in vivo* tests have been used to investigate the compatibility of poloxamers with corneal and conjunctival tissues. The *in vitro* MTT reduction cytotoxicity assay test performed using primary human corneal epithelial cells on

culture plates showed the safety of P407/P188-based formulations on corneal tissues [33]. Similarly, Asasutjarit *et al.* demonstrated the safety of a P407/P188/carbopol formulation on a rabbit corneal cell line using short-time exposure tests, where the total score of eye irritation was zero [32]. Both Fathalla *et al.* and Al Khateb *et al.* confirmed the safety of poloxamer formulations on extracted bovine eyes using a corneal erosion test [26,33]. Furrer *et al.* exploited *in vivo* confocal laser scanning ophthalmoscopy to evaluate the irritation of mouse and rabbit corneal tissues after treatment with different surfactant solutions and fluorescent dyes [58]. Eyes treated with 20% w/w P407 solution did not exhibit any significant difference in the percentage of corneal surface damage relative to those treated with saline solution.

Furthermore, Gupta and Samanta performed *in vivo* ocular tolerance tests for forskolin-loaded poloxamer-based *in situ* gels instilled in rabbit eyes [55]. A modified Draize test was used to score the inflammatory responses, where no significant macroscopic or microscopic reactions were recognised in the tested eyes relative to their contralateral eyes in the same animal.

Other *in vivo* studies investigated the compatibility of poloxamer-based formulations with retinal tissues. Hwang *et al.* examined the effects of intravitreal (IVT) injection of 20% w/w P407 on the retinal tissues of rabbit eyes. The authors reported that the formed gel block dispersed in the vitreous humour within 2 days but caused atrophic changes to the retina (Fig. 4A) [59]. Furthermore, mild cataracts developed after 2 months of injection, which could be attributed to the osmotic gradient generated by the dissolved poloxamer unimers. Similarly, Su *et al.* recommended avoiding the IVT use of P407 at a concentration >20% w/w because of its toxic effect on retina [60].

Drug delivery to the anterior segment of the eye

The merits of using poloxamer-based *in situ* gelling systems include controlled ocular drug delivery. This controlled delivery can be evaluated via *in vivo* preclinical studies that involve the instillation of drug-loaded poloxamer-based *in situ* gels versus control drug solutions, followed by investigating either the drug pharmacological effect or the drug pharmacokinetics. Some loaded drugs have a measurable pharmacological activity, such as an IOP-lowering effect, miosis, or even anti-inflammatory effects. For instance, Gupta and Samanta reported that incorporation of forskolin into a poloxamer-based *in situ* gel maintained its IOP-lowering activity in rabbit eyes for 12 h, whereas the IOP-lowering activity of forskolin suspension lasted for 7 h [55]. Results of pharmacological studies can reflect species variation in response. For example, Nomura and Hashimoto reported that latanoprost eye drops (0.005%) decreased IOP in monkeys but did not show an IOP-lowering effect in rabbits or cats [61].

By contrast, pharmacokinetic studies involve tracing the drug distribution either by measuring fluorescence or radioactivity of tagged drugs or through tissues extracted from sacrificed animals. For example, on tracing the clearance of radio-labelled timolol maleate from the rabbit eyes using gamma scintigraphy, Gupta *et al.* showed that the poloxamer-based *in situ* gelling formulations exhibited improved retention in rabbit eyes relative to timolol maleate solution [54].

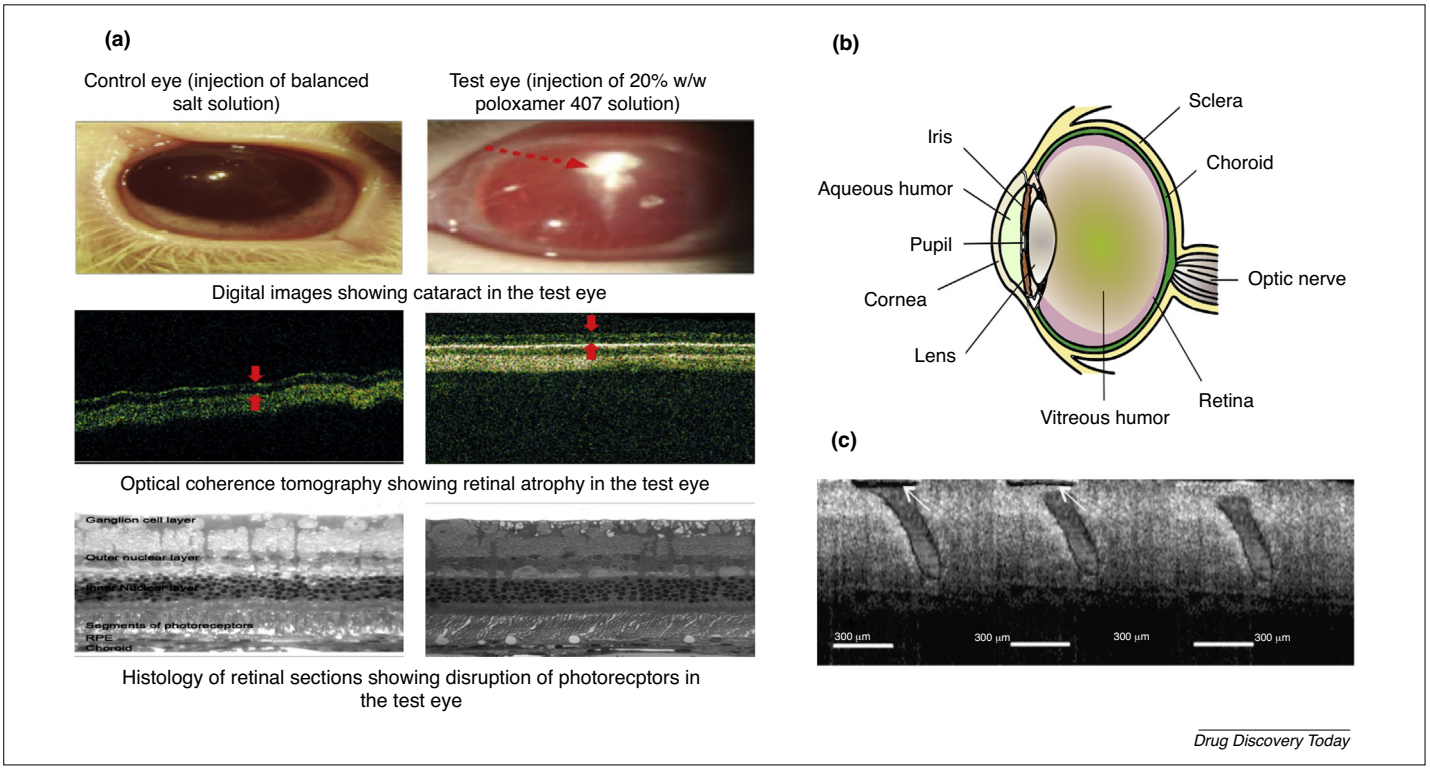

FIGURE 4

Ocular delivery of poloxamer-based formulations. (a) Rabbit eye after intravitreal injection of a poloxamer 127 formulation relative to control eye. (b) Anatomy of human eye. (c) Optical coherence tomography showing the use of 500 mm-long microneedles for intrascleral injection of a poloxamer solution. Reproduced from [59] under CC BY license (a) and with permission from [36] (c).

Other researchers have performed *in vitro* release or *ex vivo* permeation studies of drug-loaded poloxamer-based *in situ* gels intended for ocular applications. We can make use of these results to predict the *in vivo* behaviour of the formulations. However, the *in vivo* release behaviour of gel formulations is generally faster than their *in vitro* behaviour because of the shearing effects of the eyelids and eyeball motion [55].

The *in vitro* release set-ups use either dissolution testing apparatus or Franz-cell apparatus. In the dissolution testing apparatus, the *in situ* gelling formulation is placed inside a dialysis bag [54,62] or a cell covered with cellophane membrane [48,49,63], then immersed in the dissolution medium. Other studies have adopted a membrane-less technique to assess drug release and gel dissolution simultaneously, where the gel is formed separately inside a cell or tube, then directly exposed to the dissolution medium in the dissolution apparatus [55,56,63] or in a thermostatically controlled shaken test tube [41]. The dissolution medium is made of STF comprising 0.67 g NaCl, 0.2 g $NaHCO_3$, and 0.008 $CaCl_2.2H_2O$ in 100 g of purified water, maintained at 35 °C or 37 °C, and stirring rates of 20–75 rpm. The Franz-cell apparatus set-up has been used in other studies with a dialysis or cellophane membrane between the donor and receptor compartments [25,33,64,65]. The *in situ* gelling formulation is placed in the donor compartment, whereas the receptor compartment is filled with STF, phosphate buffer saline (pH 7.4), or HEPES buffer maintained at 35 °C or 37 °C. *Ex vivo* permeation studies have been performed using a similar Franz-cell set-up by replacing the semipermeable membrane with corneal tissues excised from pigs [33,65], sheep [47], rabbits [41], or goats [54].

Several studies have modelled *in vitro* drug release kinetics from poloxamer-based gels to understand the drug release mechanism from poloxamer-based gels. Moore *et al*. demonstrated that the *in vitro* release of different hydrophilic, moderately hydrophilic, and hydrophobic drugs from P407 gels exhibited zero-order kinetics, indicating that the release was controlled by the dissolution of gel irrespective of the drug nature [66]. However, Dewan *et al*. showed that the drug release followed Fickian diffusion kinetics [25]. Other studies demonstrated non-Fickian diffusion, including the diffusion of drugs out of the gel matrix and corresponding erosion of the gel [47,62]. These contradictory results indicate that the drug release mechanisms from poloxamer gels vary according to the gel composition as well as nature of the drug.

The rate of *in vitro* drug release from poloxamer gels was also affected by the gel composition; for example, Fathalla *et al*. reported that the release of ketorolac tromethamine was reduced by increasing either P188 or P407 concentrations, attributed to the increase in the formulation viscosity [33]. Several techniques have been adopted to control drug release from ocular poloxamer-based *in situ* gelling systems, as discussed here. These include the addition of polymers, copolymerisation, crosslinking, drug complexation, as well as formulation of nanosystems.

Addition of polymers

Other polymers have been added to poloxamer-based gels to provide higher gel strength and viscosity, and in turn, attain more control over drug release. These include carbomers, cellulose derivatives, and other natural polymers. Carbomers and polycarbophils are high Mwt polymers of acrylic acid (PAA), characterised by their high gelling capacity and reversible pH-dependent sol–gel transitions [1,19]. On instillation in the eye, carbomer solutions are neutralised by tear fluid buffers, enhancing ionisation, intermolecular repulsion, and subsequent gel formation [2,67]. Combined carbomer–poloxamer formulations show both temperature and pH-responsive behaviour, with enhanced ocular retention and prolonged drug release. Carbomers are commercially available with different viscosities under the trade name Carbopols® (Table 2). Carbomers polymerised in benzene are not recommended for pharmaceutical use because of safety considerations [68]. Carbomers and polycarbophils are commonly added to poloxamer formulations at concentrations 0.1–0.3% w/w to enhance viscosity and prolong drug release [32,38,43,48,49,56,69]. The addition of carbomers at concentrations ≥0.3% w/w is not recommended because it increases the formulation acidity and decreases its clarity, which can result in discomfort and interfere with the patient's vision [32,38,69]. Carbopol® 980 has poor transparency even at concentration 0.1% w/v [48]. In 2013, Insite Vision Inc. was granted a patent for Durasite®; an ophthalmic in situ gelling drug delivery platform comprising polycarbophil and a viscous mucoadhesive polymer, such as P407 [70]. Several poloxamer–polycarbophil Durasite®-based eye drops have already found their way to the market and others are in the pipeline (Table 3) [71,72].

Cellulose derivatives are also commonly added to poloxamer formulations to prolong drug release. These include MC and HPMC, which are usually added at concentrations 0.5–3% w/w depending on their Mwt and the components of the poloxamer formulation [25,39,73]. Dewan *et al*. reported that increasing either the concentration or the Mwt of MC increased the viscosity of P407 solutions and, in turn, prolonged drug release [25]. Natural

TABLE 2

Overview of most widely used polyacrylic acids in biomedical applications

Commercial name	Compendial name (USP/NF)	Polymerisation solvent	Viscosity at pH 7.5 and temperature 25 °C (cp)
Carbopol® 934 NF	Carbomer 934	Benzene	30 500–39 400[a]
Carbopol® 940 NF	Carbomer 940	Benzene	40 000–60 000[a]
Carbopol® 1342 NF	Carbomer 1342	Benzene	9500–26 500[b]
Carbopol® 974P NF	Carbomer Homopolymer B	Ethyl acetate	29 400–39 400[a]
Carbopol® 980 NF	Carbomer Homopolymer C	Cyclohexane + ethyl acetate	40 000–60 000[a]
Noveon® AA-1 polycarbophil USP	Polycarbophil	Ethyl acetate	2000–12 000[c]

[a] Viscosity of 0.5% w/w solution.
[b] Viscosity of 1% w/w solution.
[c] Viscosity of 0.2% w/w solution.

TABLE 3

Commercial products based on Durasite® platform

Commercial name	Active ingredient (w/v)	Indications	Stage of development in 2017	Distributor in USA
AzaSite®	1% Azithromycin	Bacterial conjunctivitis	FDA approved 2007	Akorn Pharmaceuticals
Besivance™	0.6% Besifloxacin	Bacterial conjunctivitis	FDA approved 2009	Bausch & Lomb
BromSite™	0.075% Bromfenac	Postoperative inflammation	FDA approved 2016	Sun Pharmaceuticals
AzaSite Plus™	1% Azithromycin + 0.1% dexamethasone	Blepharitis	Phase 3 trials	N/A
DexaSite™	0.1% Dexamethasone	Blepharitis	Phase 3 trials	N/A
ISV-101	0.01–0.04% Bromfenac	Dry eye conditions	Phase 2 trials	N/A
AzaSite Xtra™	2% Bromfenac	Blepharitis and bacterial conjunctivitis	Preclinical studies	N/A

viscosity enhancers have also been added to poloxamer formulations, including 2% w/w HA [27], 0.1% w/w alginate [62], 0.25–1% w/w chitosan [54,65], and 0.2–0.75% w/w xanthan and guar gums [64].

Copolymerisation

Copolymerisation of poloxamers with other gel-forming polymers can optimise the physicochemical properties of the in situ gel. Several trials coupling poloxamers with hydrophobic biodegradable polymers, such as polycaprolactone [74], polylactic acid [75], and oligolactides [76], offered better control over drug release without altering the thermoresponsive behaviour of poloxamers by tailoring the proportions of the copolymer blocks.

Ma *et al*. grafted PAA during its polymerisation process to P407, forming a poloxamer-g-PAA copolymer that combined the thermoresponsive behaviour of poloxamers and the mucoadhesive behaviour of PAA [77]. Increasing the proportion of acrylic acid resulted in copolymers with higher gel strength, and more prolonged *in vitro* release of gatifloxacin. In another study, Cho *et al*. coupled monoamine-terminated poloxamers with HA via a two-step reaction and showed that increasing the proportion of HA in the graft copolymer resulted in prolonging the *in vitro* release of ciprofloxacin [78]. Yu *et al*. crosslinked P407 with carboxymethyl chitosan using glutaraldehyde to control the release of nepafenac [79]. The swelling ratio of the gel in the release medium decreased with increasing the proportion of P407 in the copolymer. However, the ocular biocompatibility of the copolymer-based *in situ* gels should be investigated before *in vivo* application.

Multiblock and crosslinked poloxamers

Tailoring the physicochemical properties of P407 was achieved by chemical crosslinking between benzaldehyde-grafted and amine end-capped poloxamers [80]. Enzyme-mediated crosslinking of tyramine-conjugated poloxamers was also used to produce poloxamer-based in situ gels with tailored-release profiles [81]. Furthermore, Ahn *et al*. coupled several units of poloxamers 235 together to form biodegradable multiblock poloxamers with different in vitro release profiles [82]. These transformations maintained the reversible thermoresponsive behaviour of poloxamers with shifting of the sol–gel transition phase diagrams. By contrast, poloxamers exhibited irreversible gelation on photo-crosslinking in the presence of a photoinitiator [83]. In this study, Kwon *et al*. induced poloxamers gelation by ultraviolet (UV) irradiation after injection of the poloxamer solution in the anterior segment of rabbit eyes. The authors reported that the gel maintained its integrity for 6 months, which represents a possible potential use of poloxamers as an intraocular lens.

Drug complexation

Complexation with β-CD is a traditionally adopted technique for enhancement of drug solubility and permeation through membranes, and to improve their stability [84]. Furthermore, the release of drugs from poloxamer gels can be further controlled by complex formation, wherein Kim *et al*. demonstrated that poloxamer gels containing a rhEGF/HP-β-CD complex prolonged the in vitro release of rhEGF relative to poloxamer gels containing free rhEGF [37]. Furthermore, the rhEGF/HP-β-CD complex-loaded gel formulations exhibited relative ocular bioavailability in rabbits of 160% and 380% for 1:4 and 1:20 rhEGF:HP-β-CD complexes, respectively. Despite the promising results for in vitro release and in vivo bioavailability, researchers should be cautious about the effect of HP-β-CD on the thermoresponsive behaviour of poloxamer-based systems, as previously mentioned in this review.

Nanoformulations

Recent studies have reported various colloidal carrier systems in attempts to load poorly soluble drugs into poloxamer-based in situ thermoresponsive hydrogels. The nanoformulation approach could help improve corneal permeation and, in turn, the ocular bioavailability of these drugs. These have included drug nanocrystals, micelles, polymeric nanocapsules, as well as protein and lipid nanoparticles (NPs). Based on their surfactant properties, poloxamers can be exploited as stabilisers of the prepared nanosystems beside their primary function as gel-forming polymer. For example, Gupta *et al*. dispersed forskolin nanocrystals in a P407-polycarbophil solution and reported that the formulation maintained its stability for 6 months with no reported crystal growth [56]. Furthermore, Wang *et al*. devised a fabrication technique for a muscone-P407 nanogel by preparing an ethanolic solution of muscone containing reverse micelles of P407, followed by nitrogen drying, then reconstitution of the muscone-P407-nitrogen complex with borate buffer [62]. This reverse micelle → positive micelle technique yielded micelles that were approximately a quarter of the size of those obtained by the conventional preparation method (continuous heating of poloxamers aqueous solution). The prepared nanogels exhibited a 3.4-fold increase in corneal permeation and 6.3-fold increase in ocular bioavailability in rabbits relative to conventional muscone eye drops.

Desai and Blanchard incorporated poly(isobutyl cyanoacrylate) nanocapsules of pilocarpine into a P407/MC solution, which significantly increased the intensity and duration of miosis in rabbit eyes relative to both nanocapsule aqueous dispersion and pilocarpine *in situ* gelling formulation [85]. Similarly, Lou *et al*. incorporated curcumin-loaded albumin nanocapsules into a P407/

P188 solution, where the prepared formulation exhibited a 4.4-fold increase in ocular bioavailability in rabbits relative to curcumin suspension [86].

Lipid NP drug carriers include solid-lipid NPs (SLNs) and nanostructured lipid carriers (NLCs). SLNs comprise a solid core surrounded by a surfactant layer, whereas the core of NLCs comprises a mixture of solid and liquid lipids [87]. Hao *et al*. adopted a melt-emulsification ultrasonication technique for the preparation of SLNs, where an aqueous solution of surfactant is added to a mixture of molten lipid and traditional medicinal extract as a model lipophilic drug, followed by ultrasonication of the pre-emulsion [88]. In a study by Almeida *et al*., ibuprofen was encapsulated into NLCs using a similar technique [89]. In both studies, the obtained nanoemulsions were cooled to form drug-loaded lipid NPs and were incorporated into poloxamer-based thermoresponsive gelling systems. Confocal laser scanning microscopy showed effective penetration of SLNs across layers of rabbit cornea, while a 6-h *in vitro* release study showed a 23% release of the loaded ibuprofen from the NLC-hydrogel versus an 84% release from the corresponding ibuprofen-loaded poloxamers formulation. By contrast, liposomes cannot be deemed ideal drug carriers in poloxamer-based formulations because of the reported destabilising effect of poloxamers on the lipid bilayer membranes and the subsequent drug leakage from liposomes [90,91].

Drug delivery to the posterior segment of the eye

The posterior segment of the eye comprises the sclera, choroid, retina, and vitreous humour (Fig. 4B) [92]. Drug delivery to the posterior segment of the eye can be achieved via intraocular and periocular injections of drug solutions or suspensions for treatment of posterior eye conditions, such as age-related macular degeneration, diabetic macular edema, and posterior uveitis [92,93]. Based on the finely tunable mechanical, rheological, and thermoresponsive properties of poloxamers, poloxamer-based formulations can act as a potential vehicle for drug delivery to the posterior segment of the eye. Poloxamers can also be used for the development of *in situ*-forming depots following intraocular or periocular injection of their drug-loaded solutions, to achieve prolonged drug delivery to the posterior segment of the eye.

Intravitreal injection of poloxamer-based in situ gels

IVT injection is an invasive technique of drug delivery that is inconvenient to patients. Intravitreally injected drugs are generally slowly eliminated either by diffusion across the blood–retinal barrier (for small lipophilic molecules) or diffusion towards the aqueous humour (for all molecules) [94]. Nonetheless, the invasive nature of IVT injections poses a potential need for more extended dosing intervals. Although IVT injection of in situ gels is a promising solution for prolonged intraocular drug delivery, the safety of IVT poloxamer-based formulations is questionable, as indicated by the neuroretinal toxicity and mild cataracts that developed in rabbit eyes following IVT injection of P407 solutions of concentrations ≥20% w/w [59,60]. Further safety investigations are required in this field using in situ gelling mixtures comprising lower proportions of poloxamers with other compatible polymers. Additionally, the biodegradation of poloxamers gels is crucial to ensure their complete elimination from the ocular tissues after IVT injection. In 2008, Feng *et al*. showed that 20% w/w P407 in situ gels were almost completely degraded and discharged after 49 days of insertion in the middle ear cavity of guinea pigs [95]. These results should be confirmed through similar in vivo biodegradation studies for poloxamer-based in situ gels after their IVT injection.

Intrascleral injection of poloxamer-based in situ gels

Intrascleral injections are considered a less invasive alternative to IVT injection. However, the precise injection of formulations inside the thin scleral tissue is problematic and requires the use of hollow microneedles [96]. The use of microneedles has been thoroughly investigated in transdermal drug delivery before modestly starting to infiltrate the arena of ocular drug delivery [97]. Thakur *et al*. devised a novel way of forming intrascleral drug-releasing depots by injecting thermoresponsive in situ gelling mixtures inside the sclera using hollow microneedles 400–600-mm long (Fig. 4C) [36]. The injected mixtures comprised 12% w/w P407 and 15–20% w/w poloxamer 237, which had suitable $T_{sol–gel}$. This microneedle poloxamer-based formulation could act as a potential vehicle for the minimally invasive sustained delivery of small and large molecules to the posterior segment of the eye.

Periocular injection of poloxamer-based in situ gels

Periocular injections are considered a safe choice to avoid the risks of injuries associated with IVT injections. In contrast to IVT injection, the periocular injection of drugs is generally associated with rapid drug clearance because of high blood flow, which triggers the need for the injection of sustained-release formulations in this region. Vehanen *et al*. used ocular fluorophotometry to investigate the release of fluorescent markers from P407 in situ gelling systems injected around eyes of anaesthetised rats [98]. The authors reported that the formulations prolonged release and absorption of the markers into the vitreous humour for only 3 h. In a study by Nakatani *et al*., similar short-term ocular drug levels were obtained following the periocular injection of fluorescein isocyanate-conjugated dipeptide leucine-isoleucine incorporated in an in situ gelling solution comprising P407/sodium alginate [99]. Nevertheless, further manipulation of the mechanical properties of poloxamers formulations could improve these results.

Concluding remarks and recommendations

Poloxamer-based *in situ* gels represent potential vehicles for ocular drug delivery. Poloxamers are deemed stable against steam sterilisation and their biocompatibility with corneal tissues is well documented. P407 and P188 can be mixed in different proportions to attain a vehicle that gels at physiological temperatures. Poloxamer-based *in situ* gels have shown extended *in vitro* release of a range of drugs, with prolonged activity and increased bioavailability. However, the effect of different formulation components and tear fluids on the mechanical, rheological, and thermoresponsive properties of poloxamers must be taken into consideration.

Although poloxamer-based formulations are well exploited for commercial use to address anterior eye disease, limited work has been done to date in addressing posterior segment conditions. The retinal tolerability of poloxamer-based *in situ* gels is questionable and should be more extensively investigated before further exploitation of poloxamers via the IVT route. Biocompatibility studies should focus on the maximum safe proportion of poloxamers in IVT formulations. Moreover, biodegradation studies are required to assess the intraocular biodegradation kinetics of poloxamers

and ensure complete elimination from the injection site after delivery of their payload. By contrast, poloxamer-based *in situ* gels can be coupled with different permeation-enhancing technologies to enhance scleral permeation of the active constituent following periocular injection. This can offer promising solutions for non-invasive or minimally-invasive sustained drug delivery to the posterior segment of the eye and eliminate the need for frequent IVT injections for the treatment of posterior eye conditions.

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