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

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1 Poloxamer-based *in situ* gelling thermo-responsive systems for ocular drug delivery
2 applications

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9 **Keywords:** sol-gel transition; biocompatibility; mucoadhesion; sustained release; Pluronics

10 **Teaser:** This article discusses the advances in the use of poloxamers as *in situ* gels for ocular drug
11 delivery, highlights challenges, and recommends further possible applications

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Abstract

In situ gels have recently gained interest as ocular drug delivery vehicles as they combine the merits of easy instillation and sustained drug release. This review focuses on the use of poloxamers as *in situ* gelling systems in ocular drug delivery due to its thermo-responsive gelling behaviour, biocompatibility, and ease of sterilisation. Furthermore, the sol-gel transition temperature, mucoadhesive properties, and drug release profiles of poloxamers-based *in situ* gels can be finely tuned, hence effectively used as vehicles for delivering of small and large drug molecules in treating both anterior and posterior segments of the eye diseases. Poloxamers-based ocular products have already found their way to the pharmaceutical market yet remain a potential arena for further investigation and commercial exploitation.

1 **Introduction**

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

12 Hydrogels are a class of hydrophilic three-dimensional network, which can absorb and retain large
13 amount of water, making them important materials for drug delivery [4]. They can be used as drug
14 delivery vehicles to the eye in order to prolong precorneal retention time and in turn, improve ocular
15 bioavailability. *In situ* gelling systems are solutions which undergo transition into semisolid gels in
16 response to physiological stimuli, such as body temperature, physiological pH, and ionic strength of
17 the biological fluids [5,6]. Thus, instillation of *in situ* gelling systems in the eye combines the merits
18 of accurate dosing and easy administration of eye drops, together with prolonged retention in the eye
19 and sustained drug delivery [5]. Furthermore, *in situ* gelling systems can act as promising vehicles
20 for intraocular and periocular injections, where they can create depots after injection into the vitreous
21 humor or in periocular tissues to provide sustained drug release to the posterior segment of the eye
22 [7].

23 The thermo-responsive gelling systems are polymeric solutions which undergo sol-gel transition in
24 response to temperature change. The lower critical solution temperature (LCST) is the minimum sol-
25 gel transition temperature ($T_{sol-gel}$) of the polymer on its temperature-concentration phase diagram,

1 and it depends on the interactions between water molecules and different hydrophilic/hydrophobic
2 segments in the polymeric chain [6,8]. Several natural and synthetic polymers exhibit thermo-
3 responsive gelling behaviour at temperatures close to body temperature, hence, they can be used as
4 injectable solutions or eye drops to achieve sustained drug delivery. For example, the aqueous
5 solutions of methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) exhibit an initial
6 drop in viscosity upon heating, followed by solidification into hydrogels on continuous heating
7 [9,10]. Chitosan-based thermo-responsive hydrogels were introduced by Chenite *et al.* as
8 biocompatible sustained drug delivery systems [11]. It successfully provided sustained delivery of
9 latanoprost and ferulic acid in rabbit eyes [12,13]. Miyazaki *et al* showed that enzyme-degraded
10 xyloglucan solution loaded with pilocarpine hydrochloride exhibited thermo-responsive gelling
11 behaviour in rabbit eye and increased the duration of the miosis [14]. Poly(N-isopropylacrylamide)
12 (pNIPAAm) is a thermo-responsive polymer having LCST of around 32°C, which can be tuned by
13 grafting hydrophilic monomers [15]. For instance, Derwent *et al* and Egbu *et al.* exploited pNIPAAm
14 crosslinked with poly(ethylene glycol) diacrylate (PEGDA) with or without hyaluronic acid (HA)
15 for delivery of proteins to the posterior segment of eye, where the hydrogels displayed LCSTs
16 ranging from 31 - 36°C, depending on the PEGDA content [16,17]. Gao *et al* synthesized poly-(DL-
17 lactic acid-co-glycolic acid)-poly(ethylene glycol) (PLGA-PEG) copolymer, which exhibited a T_{sol-}
18 $_{gel}$ of 32°C and was used as *in situ* gel for ocular delivery of dexamethasone acetate [18].

19 Poloxamers are synthetic polymers which exhibit a thermo-responsive behaviour, with a finely
20 tunable $T_{sol-gel}$, hence, they are extensively used for several pharmaceutical and biomedical
21 applications [2,19,20]. They are commercially available as Pluronic[®], Kolliphors[®], and Lutrols[®]
22 [21]. Poloxamer 407 (P407) and poloxamer 188 (P188) are among the most commonly used
23 poloxamers in ocular drug delivery as a result of their good solubility in water, clarity of their
24 aqueous solutions, concentration-dependent viscosity, shear-thinning behaviour of their aqueous
25 solutions, and their safety to the ocular tissues. This review article is primarily concerned in

1 presenting the physicochemical properties of poloxamers, particularly their thermo-responsive
2 behaviour, and their potential applications in ocular drug delivery for both anterior and posterior
3 segments of the eye.

4 **Physicochemical properties of poloxamers-based *in situ* ocular gels**

5 *Chemical structure of poloxamers*

6 Poloxamers are non-ionic surfactants having triblock copolymer structure consisting of two
7 hydrophilic poly(ethylene oxide) (PEO) blocks with a hydrophobic poly(propylene oxide) (PPO)
8 block (**Figure 1A**) [2,19]. Their different PEO:PPO proportions contribute to their variable
9 physicochemical properties. Poloxamers have a unique nomenclature system composed of three
10 digits, where the first two digits represent the approximate molecular weight (Mwt) of the PPO block
11 divided by 100, while the third digit represents the approximate weight percentage of the PEO
12 divided by 10. On the other hand, Pluronics® are nominated by a letter representing their physical
13 state followed by a three-digit number which depends on the PEO:PPO weight fraction. Pluronics®
14 are given a letter (L) for liquid, (P) for paste, and (F) for flakes. The first two digits represent the
15 approximate Mwt of the PPO block divided by 300, while the third digit represents the approximate
16 weight percentage of the PEO divided by 10 [21]. **Table 1** presents examples of different poloxamers
17 and their corresponding commercially available Pluronics® as per the manufacturer.

18 *Thermo-responsive behaviour of poloxamers*

19 Due to their surfactant properties, poloxamers molecules self-associate forming micelles at a certain
20 concentration known as critical micelle concentration (CMC). During micelle formation, the PPO
21 groups interact together *via* van der Waals forces to form the hydrophobic micelles core, while the
22 PEO groups occupy the micelles shell interacting with water molecules by hydrogen bonds [3].
23 Temperature rise favours interaction 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, poloxamers micelles aggregate together at a certain temperature and the system fluidity decreases abruptly leading to gel formation. This process is reversible as cooling converts the gel back to its original sol state (**Figure 1B**) [22]. In the past three decades, the thermo-responsive behaviour of poloxamers has been thoroughly investigated with respect to the development of sensitive and precise techniques for determination of the $T_{sol-gel}$, as well as investigation of different molecular and formulation variables affecting their thermo-responsive gelling behaviour.

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

Different techniques have been developed for the determination of the $T_{sol-gel}$. The simplest method is test tube inversion where a test tube containing the sample is repeatedly tilted in a gradually heated water bath and recording the temperature at which no flow occurs (**Figure 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 a 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 determination of the $T_{sol-gel}$ include scanning of the UV-visible absorbance of polymeric solutions at 500 nm with a gradual increase of temperature, where an absorbance peak is observed at the gelation point [25]. Similarly, dynamic light scattering (DLS) can trace the aggregation of micelles through measuring the hydrodynamic diameters in polymeric solution at different temperatures and concentrations [26,27]. Furthermore, the gelation process is associated with a secondary endothermic peak which can be detected using micro-differential scanning calorimetry (micro-DSC) (**Figure 2B**) [28]. Either UV-spectroscopy, DLS, or micro-DSC yield

1 accurate results of the $T_{sol-gel}$, yet, they do not give insight about changes in the rheological
2 behaviours at the $T_{sol-gel}$.

3 Rheological measurements can be done using temperature-controlled rotational viscometers, where
4 the sample is heated slowly at a constant shear rate and the $T_{sol-gel}$ is determined as the temperature
5 at which the sample exhibits an abrupt increase in viscosity [3]. However, the rate of temperature
6 increase has a critical effect on the accuracy of this method, where rapid temperature increase may
7 result in recording a higher false $T_{sol-gel}$, because of the time elapsed during gel-network formation
8 and the subsequent increase in sample viscosity. The $T_{sol-gel}$ can be determined more accurately by
9 operating the rotational viscometer in oscillation mode, where the sample temperature is increased
10 slowly with concurrent measurement of both the elastic (storage, G') and the viscosity (loss, G'')
11 moduli. The elastic modulus is proportional to the energy stored and returned on oscillation, while
12 the viscosity modulus is proportional to the energy dissipated in friction. Therefore, $G' > G''$ in
13 predominantly elastic solids, whereas $G'' > G'$ in predominantly viscous liquids. The $T_{sol-gel}$ can be
14 determined as the point at which G' and G'' intersect (**Figure 2C**) [27,29,30].

15 *Optimization of $T_{sol-gel}$ of poloxamers solutions for ocular use*

16 The optimum *in situ* gelling formulation should have a $T_{sol-gel}$ fairly above room temperature and
17 below the precorneal temperature (25 – 34.5°C), ideally $30 \pm 2^\circ\text{C}$ [31]. However, P407 aqueous
18 solutions exhibit unsatisfactory $T_{sol-gel}$ of values below room temperature at concentrations 20 – 30%
19 w/w, whereas P188 aqueous solutions have $T_{sol-gel} > 40^\circ\text{C}$ at the same concentrations. Therefore,
20 mixtures of both poloxamers are commonly used in different proportions in order to yield desired
21 $T_{sol-gel}$ [32]. Increasing the proportion of P407 results in decreasing the $T_{sol-gel}$, due to the increased
22 number of micelles, which decreases the energy needed for the endothermic micellar crystallisation,
23 resulting in subsequent gelation at lower temperature [28]. On the other hand, increasing the
24 proportion of the more hydrophilic P188 disrupts P407 micelles, which results in increasing the

energy required by P407 to undergo hydrophobic interaction with a subsequent increase in the $T_{sol-gel}$ [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 the $T_{sol-gel}$ [34,35]. At these concentrations, the gel formation can be explained by the jamming effect of micelles rather than micellar crystallisation [28]. Formulations containing P188 at concentrations $\geq 20\%$ w/w possess 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 the following equation for calculation of the $T_{sol-gel}$ of P407/P188 mixtures [34]:

$$T_{sol-gel} = 87.13 - 2.65 C_{407} - 0.41 C_{188} \quad \text{Equation (1)}$$

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

In 2010, Qian *et al* modified the previous equation as following [35]:

$$T_{sol-gel} = 277.08 C_{407}^2 - 737.03 C_{188}^2 - 409.03 C_{407} + 1.07 C_{188} + 566.09 C_{407} C_{188} + 97.78$$

Equation (2)

Before making optimum poloxamer preparation, the effect of other formulation components on the $T_{sol-gel}$ must be taken into consideration. Drug addition will influence the $T_{sol-gel}$ of the poloxamers formulation, e.g. 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 P407/P188 solution resulted in increasing the $T_{sol-gel}$ by 1°C [37]. Furthermore, addition of Tween 80 at concentrations 0.5 – 1.5% w/w resulted in raising of the $T_{sol-gel}$ of 20% w/w P407 solutions by 6 – 11°C [38]. On the other hand, Krtalić *et al.* showed that small 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 poloxamers solution resulted in decreasing the $T_{sol-gel}$ by 2 – 3°C [35,39]. Alexandridis

1 and Holzwarth also studied the effect of different salts on the $T_{sol-gel}$ and explained this effect through
2 salting-out phenomenon which is correlated to ionic radius of the added salts and their solvation heat
3 [40].

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

13 *Mechanical and rheological properties of poloxamers-based gels*

14 The mechanical and rheological properties of poloxamers-based gels are closely correlated and
15 directly affect the gel retention time within the eye, where the relatively weak mechanical strength
16 and low viscosity of poloxamers gels may contribute to their relatively rapid erosion [42].
17 Nonetheless, high mechanical strength and viscosity are generally unfavourable due to the difficulty
18 of application and patient inconvenience [43]. Therefore, optimum mechanical strength and viscosity
19 provide a balance between easy administration and long retention time within the eye [24]. The
20 mechanical properties of poloxamers-based gels can be assessed using TA-XT texture analyser at
21 physiological temperature, where the gel hardness was measured as the force of gel compression,
22 expressing the applicability of the gel to the eye, while the gel compressibility was measured as the
23 work required to deform the gel expressing its spreadability on the ocular tissues [24,43,44]. On the

1 other hand, the gel viscosity at physiological temperature can be measured using rotational
2 viscometers.

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

14 *Mucoadhesive properties of poloxamers-based gels*

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

1 temperature. Mucoadhesive force can also be measured *in vitro* by using TA-XT texture analyser,
 2 where mucin discs were attached to the probe using double-sided adhesive tape and brought in
 3 contact with the gel formulation, followed by measuring the force required to detach the mucin disc
 4 from the gel [30,33]. Similarly, the mucoadhesive force of the gel to the corneal tissues was measured
 5 using peel test method, where poloxamers gel was pressed against excised rabbit cornea using
 6 Instron[®] testing machine, followed by raising the probe to measure the detachment force between
 7 the gel and the corneal tissues [37]. Mucoadhesive force can be also determined by evaluation of the
 8 rheological synergism between mucin and the polymer formulation, where viscosities of mucin (η_m),
 9 polymer formulation (η_p), and mixture of both (η_t) were measured at a certain shear rate, and were
 10 used to calculate the viscosity component of mucoadhesion (η_b) using the following equation
 11 [26,27]:

$$12 \quad \eta_b = \eta_t - (\eta_m + \eta_p) \quad \text{Equation (3)}$$

13 The mucoadhesive force (F_b) can be calculated using the following equation [26,27]:

$$14 \quad F_b = \eta_b \cdot \gamma' \quad \text{Equation (4)}$$

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

16 P407/P188 mixtures have certain mucoadhesive properties, e.g. Fathalla *et al.* showed that the work
 17 of adhesion increased with increasing the concentration of P188 up to 15% w/v, whereas it decreased
 18 on increasing P407 above 23% w/v [33]. Other polymers may be added to improve
 19 mucoadhesiveness, such as chitosan (1% w/w) which increased the mucoadhesive force of 18% w/w
 20 P407 gels by 26% [30]. Addition of 0.1% w/v Carbopol[®] 1342 resulted in 3-fold increase in the
 21 mucoadhesive force of the poloxamers mixture composed of 21% w/v P407 and 5% w/v [48].
 22 Similarly, Cao *et al.* showed that addition of 0.3% w/v Carbopol[®] 974 resulted in 2.3-fold
 23 improvement in the mucoadhesive force which was associated with a slight decrease in the $T_{sol-gel}$
 24 [49]. Gelrite and low Mwt HA (150 kDa) were also shown to improve the mucoadhesive force of

1 P407 gels [27,47]. This was associated with increasing the gel-strength and a subsequent decrease in
2 the $T_{sol-gel}$. In contrast, the addition of 0.2% w/w high Mwt sodium hyaluronate (1200 kDa) to the
3 poloxamers formulation hindered micelle formation, resulting in decreased gel-strength, hence the
4 precorneal retention time was not improved [34]. Therefore, the probable adverse effects of added
5 polymers on the mechanical, rheological, and thermo-responsive properties of the formulation must
6 be thoroughly investigated.

7 **Ocular applications of poloxamers-based *in situ* gels**

8 *Historical overview*

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

22 In the late 1980s, Saettone *et al.* investigated the use of different poloxamers as solubilisers for
23 tropicamide [52]. The authors shortly described the gel-forming property of poloxamers on
24 instillation in the eye and excluded the *in situ* gelling formulation as a result of – what was

1 considered, as its undesirable rheological behaviour. In another study published in 1989, Saettone *et*
2 *al.* exploited the solubilising and *in situ* thermo-responsive gelling properties of poloxamers in
3 formulation of forskolin solutions for ocular use, where the forskolin gelling solution exhibited
4 prolonged intraocular pressure (IOP) lowering activity in rabbit model relative to forskolin
5 suspension [53]. It is worthy of mentioning that the improvement in solubility of both tropicamide
6 and forskolin did not result in subsequent improvement of ocular bioavailability due to binding of
7 drugs inside poloxamers micelles.

8 *Effect of sterilisation*

9 The major challenge facing formulation of ocular gels is their sterilisation. Most polymers cannot
10 withstand common sterilisation techniques, where the application of heat, radiation, or chemical
11 sterilisation may trigger side reactions leading to loss of the polymer gelling properties. Different
12 studies addressed the stability of poloxamers gels to steam sterilisation, where *in situ* gels composed
13 of mixtures of P407, P188, Tween 80, chitosan, and Carbopol were deemed stable against autoclave
14 sterilisation at 121°C and 15 psi for 20 min concerning their $T_{sol-gel}$ and flow behaviour [32,38,54].
15 On the other hand, thermosensitive drugs can be sterilised by membrane filtration of their solutions
16 followed by crystallisation and incorporation into the steam-sterilised polymer formulation under
17 aseptic conditions [55,56].

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

24

Ocular biocompatibility of poloxamers-based gels

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

A number of *in vitro*, *ex vivo*, and *in vivo* tests have been adopted for investigation of the compatibility of poloxamers with corneal and conjunctival tissues. The *in vitro* MTT reduction cytotoxicity assay test which was 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 P407/P188/Carbopol formulation on rabbit corneal cell line using short time exposure test [32]. Both Fathalla *et al.* and Al Khateb *et al.* confirmed the safety of poloxamers formulations on extracted bovine eyes using corneal erosion test [26,33]. Furrer *et al.* exploited *in vivo* confocal laser scanning ophthalmoscopy for evaluation of irritation of mice and rabbit corneal tissues after treatment with different surfactant solutions and fluorescent dye [58]. Eyes treated with 20% w/w P407 solution did not exhibit any significant difference in % 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 the rabbit eyes [55]. Modified Draize test was used for scoring of the inflammatory responses, where no significant macroscopic or microscopic reactions were recognized in the tested eyes relative to their contralateral eyes in the same animal. Similarly, Asasutjarit *et al.* used a modified Draize test to evaluate the *in vivo* ocular toxicity of P407/P188/Carbopol in rabbits eyes, where the total score of eye irritation was equal to zero [32].

Other *in vivo* studies investigated the compatibility of poloxamers-based formulation with retinal tissues. Hwang *et al.* examined the effects of intravitreal injection of 20% w/w P407 on the retinal tissues of rabbits eyes. The authors reported that the formed gel block dispersed in the vitreous humor

1 within 2 days but caused atrophic changes to the retina (**Figure 4A**) [59]. Furthermore, mild cataract
2 developed after 2 months of injection which could be attributed to the generated osmotic gradient by
3 dissolved poloxamers unimers. Similarly, Su *et al.* recommended avoiding the intravitreal use of
4 P407 at a concentration > 20% w/w due to its toxic effect on retina [60].

5 *Drug delivery to the anterior segment of the eye*

6 The merits of using poloxamers-based *in situ* gelling system include the achievement of controlled
7 ocular drug delivery. This controlled delivery can be evaluated *via in vivo* preclinical studies which
8 involve the instillation of drug-loaded poloxamers-based *in situ* gels *vs.* control drug solutions,
9 followed by investigating either the drug pharmacological effect or the drug pharmacokinetics. Some
10 loaded drugs have a measurable pharmacological activity such as IOP-lowering effect, miosis, or
11 even anti-inflammatory effect. For instance, Gupta and Samanta reported that incorporation of
12 forskolin into poloxamers-based *in situ* gel maintained its IOP-lowering activity in rabbits eyes for
13 12 hours, whereas the IOP-lowering activity of forskolin suspension lasted for 7 hours only [55].
14 The results of pharmacological studies may suffer from species variation in response. For example,
15 Nomura and Hashimoto reported that latanoprost eye drops (0.005%) decreased IOP in monkeys but
16 did not show an IOP-lowering effect in rabbits or cats [61].

17 On the other hand, pharmacokinetic studies involve tracing of drug distribution either through
18 measuring fluorescence or radioactivity of tagged drugs or through tissue extraction of sacrificed
19 animals. For example, on tracing the clearance of radio-labelled timolol maleate from the rabbit eyes
20 using gamma scintigraphy, Gupta *et al.* showed that the poloxamers-based *in situ* gelling
21 formulations exhibited improved retention in rabbits eyes relative to timolol maleate solution [54].

22 Many other researchers performed *in vitro* release or *ex vivo* permeation studies for drug-loaded
23 poloxamers-based *in situ* gels intended for ocular applications. We can make use of these studies
24 results to roughly expect the *in vivo* behaviour of the formulations. However, it should be taken into

1 account that the *in vivo* release behaviour of gel formulations is generally faster than their *in vitro*
2 behaviour due to the shearing effects of the eyelids and eyeball motion [55].

3 The *in vitro* release set-ups used either dissolution testing apparatus or Franz-cell apparatus. In the
4 dissolution testing apparatus, the *in situ* gelling formulation was placed inside a dialysis bag [54,62]
5 or a cell covered with cellophane membrane [48,49,63], then immersed in the dissolution medium.
6 Other studies adopted a membrane-less technique to assess drug release and gel dissolution
7 simultaneously, where the gel is formed separately inside a cell or tube, then directly exposed to the
8 dissolution medium in the dissolution apparatus [55,56,63] or in a thermostatically-controlled shaken
9 test tube [41]. The dissolution medium was made of STF composed of 0.67 g NaCl, 0.2 g NaHCO₃,
10 and 0.008 CaCl₂·2H₂O in 100 g of purified water, maintained at temperature 35 or 37°C, and stirring
11 rates 20-75 rpm. Franz-cell apparatus set-up was used in other studies with a dialysis or cellophane
12 membrane between the donor and receptor compartments [25,33,64,65]. The *in situ* gelling
13 formulation was placed in the donor compartment while the receptor compartment was filled with
14 STF, phosphate buffer saline (pH 7.4), or HEPES buffer maintained at 35 or 37°C. *Ex vivo*
15 permeation studies were performed using similar Franz-cell set-up by replacing the semipermeable
16 membrane with corneal tissues excised from pigs [33,65], sheep [47], rabbits [41], or goats [54].

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

1 The rate of *in vitro* drug release from poloxamers gels was also affected by the gel composition, e.g.
2 Fathalla *et al.* reported that the release of ketorolac tromethamine was reduced with increasing either
3 P188 or P407 concentrations is attributed to the increase in the formulation viscosity [33]. Several
4 techniques were adopted for control of drug release from ocular poloxamers-based *in situ* gelling
5 systems as will be discussed in this section. These include the addition of polymers,
6 copolymerisation, crosslinking, drug complexation, as well as formulation of nanosystems.

7 (i) Addition of polymers

8 Other polymers were added to poloxamers-based gels to provide higher gel strength and viscosity,
9 and in turn, attain more control over drug release. These included carbomers, cellulose derivatives,
10 and other natural polymers. Carbomers and polycarbophils are high Mwt polymers of acrylic acid
11 (PAA), characterised by their high gelling capacity and their reversible pH-dependent sol-gel
12 transitions [1,19]. On instillation in the eye, carbomers solutions get neutralised by tear fluid buffers,
13 enhancing ionisation, intermolecular repulsion, and subsequent gel formation [2,67]. Combined
14 carbomers-poloxamers formulations possess both temperature and pH-responsive behaviour with
15 enhanced ocular retention and prolonged drug release. Carbomers are commercially available with
16 different viscosities under trade name Carbopols[®] as shown in **Table 2**. The carbomers polymerised
17 in benzene are not recommended for pharmaceutical use due to safety considerations [68].
18 Carbomers and polycarbophils are commonly added to poloxamers formulations at concentrations
19 0.1 – 0.3% w/w to enhance viscosity and prolong drug release [32,38,43,48,49,56,69]. Addition of
20 carbomers at concentration $\geq 0.3\%$ w/w is not recommended because it increases the formulation
21 acidity and decreases its clarity, which may result in discomfort and interfere with the patient's vision
22 [32,38,69]. Carbopol[®] 980 has poor transparency even at concentration 0.1% w/v [48]. In 2013,
23 Insite Vision Inc. was granted a patent for Durasite[®]; an ophthalmic *in situ* gelling drug delivery
24 platform composed of polycarbophil and a viscous mucoadhesive polymer such as P407 [70].

1 Several poloxamer-polycarbophil Durasite[®] based eye drops have already found their way to the
2 market and others are in the pipeline (**Table 3**) [71,72].

3 Cellulose derivatives are also commonly added to poloxamers formulations to prolong drug release.
4 These include MC and HPMC which are usually added at concentrations 0.5 – 3% w/w depending
5 on their Mwt and the components of the poloxamers formulation [25,39,73]. Dewan *et al.* reported
6 that increasing either the concentration or the Mwt of MC resulted in increasing the viscosity of P407
7 solutions and in turn, more prolonged drug release [25]. Natural viscosity enhancers were also added
8 to poloxamers formulations, including 2% w/w HA [27], 0.1% w/w alginate [62], 0.25 – 1% w/w
9 chitosan [54,65], and 0.2 - 0.75% w/w xanthan and guar gums [64].

10 (ii) Co-polymerisation

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

16 Ma *et al.* grafted PAA during its polymerisation process to P407 forming a poloxamer-g-PAA
17 copolymer, which combined the thermo-responsive behaviour of poloxamers and the mucoadhesive
18 behaviour of PAA [77]. Increasing the proportion of acrylic acid resulted in copolymers with higher
19 gel strength, and more prolonged *in vitro* release of gatifloxacin. In another study, Cho *et al.* coupled
20 monoamine-terminated poloxamers with HA *via* a 2-step reaction and showed that increasing the
21 proportion of HA in the graft copolymer resulted in prolonging the *in vitro* release of ciprofloxacin
22 [78]. Yu *et al.* crosslinked P407 with carboxymethyl chitosan using glutaraldehyde for controlling
23 the release of nepafenac [79]. The swelling ratio of the gel in the release medium decreased with

1 increasing the proportion of P407 in the copolymer. The ocular biocompatibility of the copolymers-
2 based *in situ* gels should be investigated prior to *in vivo* application.

3 (iii) Multiblock and crosslinked poloxamers

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

15 (iv) Drug Complexation

16 Complexation with β -CD is a traditionally adopted technique for enhancement of drugs solubility,
17 permeation through membranes, and improving their stability [84]. Furthermore, the release of drugs
18 from poloxamers gels could be further controlled by complex formation, where Kim *et al.*
19 demonstrated that poloxamers gels containing rhEGF/HP- β -CD complex prolonged the *in vitro*
20 release of rhEGF relative to poloxamers gels containing free rhEGF [37]. Furthermore, the
21 rhEGF/HP- β -CD complex-loaded gel formulations exhibited relative ocular bioavailability in rabbits
22 of approximately 160% and 380% for 1:4 and 1:20 rhEGF:HP- β -CD complexes, respectively.
23 Despite the promising results for *in vitro* release and *in vivo* bioavailability, researchers should be

1 cautious about the effect of HP- β -CD on the thermo-responsive behavior of poloxamers-based
2 systems as previously mentioned in this review.

3 (v) Nano-formulations

4 Recent studies developed various colloidal carrier systems in attempts to load poorly soluble drugs
5 into poloxamers-based *in situ* thermo-responsive hydrogels. The nano-formulation approach would
6 help improve corneal permeation and in turn, ocular bioavailability of these drugs. These included
7 drug nanocrystals, micelles, polymeric nanocapsules, as well as protein and lipid nanoparticles.
8 Based on their surfactant properties, poloxamers can be exploited as stabilisers of the prepared
9 nanosystems beside their primary function as gel-forming polymer. For example, Gupta *et al.*
10 dispersed forskolin nanocrystals in P407-polycarbophil solution and reported that the formulation
11 maintained its stability for 6 months with no reported crystal growth [56]. Furthermore, Wang *et al*
12 devised a fabrication technique for muscone-P407 nanogel, by preparing an ethanolic solution of
13 muscone containing reverse micelles of P407, followed by nitrogen drying, then reconstitution of
14 the muscone-P407-nitrogen complex with borate buffer [62]. This reverse micelle $\rightarrow$ positive
15 micelle technique yielded micelles of the approximately $\frac{1}{4}$ size of those obtained by the conventional
16 preparation method (continuous heating of poloxamers aqueous solution). The prepared nanogels
17 exhibited a 3.4-fold increase in corneal permeation and 6.3-fold increase in ocular bioavailability in
18 rabbits relative to conventional muscone eye drops.

19 Desai and Blanchard incorporated poly(isobutyl cyanoacrylate) nanocapsules of pilocarpine into
20 P407/MC solution, which significantly increased the intensity and duration of miosis in rabbit eyes
21 relative to both nanocapsules aqueous dispersion and pilocarpine *in situ* gelling formulation [85].
22 Similarly, Lou *et al.* incorporated curcumin-loaded albumin nanocapsules into P407/P188 solution,
23 where the prepared formulation exhibited a 4.4-fold increase in ocular bioavailability in rabbits
24 relative to curcumin suspension [86].

1 Lipid nanoparticle drug carriers include solid-lipid nanoparticles (SLNs) and nanostructured lipid
2 carriers (NLCs). SLNs are composed of a solid core surrounded by a surfactant layer, while the core
3 of NLCs is composed of mixture of solid and liquid lipids [87]. Hao *et al.* adopted melt-
4 emulsification ultrasonication technique for preparation of SLNs, where an aqueous solution of
5 surfactant is added to a mixture of molten lipid and traditional medicinal extract as a model lipophilic
6 drug, followed by ultrasonication of the pre-emulsion [88]. In another study by Almeida *et al.*,
7 ibuprofen was encapsulated into NLCs using similar technique [89]. In both studies, the obtained
8 nanoemulsions were cooled down to form drug-loaded lipid nanoparticles and were incorporated
9 into poloxamers-based thermo-responsive gelling systems. Confocal laser scanning microscopy
10 showed effective penetration of SLNs across layers of rabbit cornea, while a 6-hour *in vitro* release
11 study showed 23% release of the loaded ibuprofen from the NLC-hydrogel *vs* 84% release from the
12 corresponding ibuprofen-loaded poloxamers formulation. On the other hand, liposomes cannot be
13 deemed ideal drug carriers in poloxamer-based formulations due to the reported destabilising effect
14 of poloxamers on the lipid bilayer membranes and the subsequent drug leakage from liposomes
15 [90,91].

16 *Drug delivery to the posterior segment of the eye*

17 The posterior segment of the eye is composed of sclera, choroid, retina and vitreous humor (**Figure**
18 **4B**) [92]. Drug delivery to the posterior segment of the eye can be achieved through intraocular and
19 periocular injections of drug solutions or suspensions for treatment of posterior eye conditions such
20 as age-related macular degeneration, diabetic macular edema, and posterior uveitis [92,93]. Based
21 on the finely tunable mechanical, rheological and thermo-responsive properties of poloxamers,
22 poloxamers-based formulations can act as a potential vehicle for drug delivery to the posterior
23 segment of the eye. Poloxamers can be used for the development of *in situ* forming depots following

1 intraocular or periocular injection of their drug-loaded solutions, to achieve prolonged drug delivery
2 to the posterior segment of the eye.

3 *(i) Intravitreal injection of poloxamers-based in situ gels*

4 Intravitreal injection is an invasive technique of drug delivery suffering from the patient
5 inconvenience. Intravitreally injected drugs are generally slowly eliminated either through diffusion
6 across blood-retinal barrier (for small lipophilic molecules) or diffusion towards the aqueous humor
7 (for all molecules) [94]. Nonetheless, the invasive nature of the intravitreal injection poses a potential
8 need for more extended dosing intervals. Although intravitreal injection of *in situ* gels is a promising
9 solution for prolonged intraocular drug delivery, the safety of intravitreal poloxamers-based
10 formulations is questionable, as reported by the neuroretinal toxicity and mild cataract developed in
11 rabbit eyes following intravitreal injection of P407 solutions of concentrations $\geq 20\%$ w/w [59,60].
12 Further safety investigations are required in this field using *in situ* gelling mixtures composed of
13 lower proportions of poloxamers with other compatible polymers. Additionally, the biodegradation
14 of poloxamers gels is crucial to ensure their complete elimination from the ocular tissues after
15 intravitreal injection. In 2008, Feng *et al* showed that 20% w/w P407 *in situ* gels were almost
16 completely degraded and discharged after 49 days of insertion in middle ear cavity of guinea pigs
17 [95]. These results should be confirmed through similar *in vivo* biodegradation studies for
18 poloxamers-based *in situ* gels after their intravitreal injection.

19 *(ii) Intrasccleral injection of poloxamers-based in situ gels*

20 Intrasccleral injection is considered as a less invasive alternative to intravitreal injection. However,
21 precise injection of formulations inside the thin scleral tissue is problematic and requires the use of
22 hollow microneedles [96]. The use of microneedles has been thoroughly investigated in transdermal
23 drug delivery before modestly starting to infiltrate the arena of ocular drug delivery [97]. Thakur *et*
24 *al* devised a novel way of forming intrasccleral drug-releasing depots by injecting thermo-responsive

1 *in situ* gelling mixtures inside the sclera using hollow microneedles of length 400 – 600 μm (**Figure**
2 **4C**) [36]. The injected mixtures were composed of 12% w/w P407 and 15 – 20% w/w poloxamer
3 237, which had suitable $T_{sol-gel}$. This microneedle poloxamers-based formulation can act as a
4 potential vehicle for minimally-invasive sustained delivery of small and large molecules to the
5 posterior segment of the eye.

6 (iii) *Periocular injection of poloxamers-based in situ gels*

7 The periocular injection is considered as a safe choice to avoid the risks of eye injuries associated
8 with intravitreal injections. In contrast to intravitreal injection, periocular injection of drugs is
9 generally associated with rapid drug clearance due to high blood flow, which triggers the need for
10 injection of sustained release formulations in this region. Vehanen *et al.* investigated the release of
11 fluorescent markers from P407 *in situ* gelling systems injected around eyes of anaesthetized rats
12 using ocular fluorophotometry [98]. The study reported that the formulations prolonged release and
13 absorption of the markers into the vitreous humor for only 3 h. In another study by Nakatani *et al.*,
14 similar short-term ocular drug levels were obtained on periocular injection of fluorescein isocyanate
15 conjugated dipeptide leucine-isoleucine incorporated in an *in situ* gelling solution composed of
16 P407/sodium alginate [99]. Nevertheless, further manipulation of the mechanical properties of
17 poloxamers formulations can improve these results.

18 **Conclusions and recommendations**

19 Poloxamers-based *in situ* gels represent potential vehicles for ocular drug delivery. Poloxamers are
20 deemed stable against steam sterilisation and their biocompatibility with corneal tissues is well-
21 documented. P407 and P188 can be mixed in different proportions to attain a vehicle that gels at
22 physiological temperature. Poloxamers-based *in situ* gels showed extended *in vitro* release of a wide
23 range of drugs, with prolonged activity and increased bioavailability. However, the effect of different

1 formulation components and tear fluids on the mechanical, rheological, and thermo-responsive
2 properties of poloxamers must be taken into consideration.

3 Although poloxamers-based formulations are well-exploited for commercial use to address anterior
4 eye disease, limited work has been done to date in addressing posterior segment conditions. The
5 retinal tolerability of poloxamers-based *in situ* gels is questionable and should be more extensively
6 investigated before further exploitation of poloxamers via intravitreal route. Biocompatibility studies
7 should focus on the maximum safe proportion of poloxamers via the intravitreal formulation.
8 Moreover, biodegradation studies are required to assess the intraocular biodegradation kinetics of
9 poloxamers and ensure complete elimination of poloxamers from their injection site after delivery
10 of their payload. On the other hand, poloxamers-based *in situ* gels can be coupled with different
11 permeation-enhancing technologies to enhance scleral permeation of the active constituent following
12 periocular injection. This can offer promising solutions for non-invasive sustained drug delivery to
13 the posterior segment of the eye and eliminate the need for frequent intravitreal injections for
14 treatment of posterior eye conditions.

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1 **Tables Legends:**

2 **Table 1.** List of commonly marketed poloxamers as provided by the manufacturer.

3 **Table 2.** Overview of most widely used polyacrylic acids in biomedical applications as provided by
4 the manufacturer.

5 **Table 3.** Commercial products based on Durasite[®] platform.

6

7 **Figures legends:**

8 **Figure 1** Schematic diagram of (A) chemical structure of poloxamers; (B) molecular phase changes
9 exhibited on changing temperature.

10 **Figure 2** Methods for determination of sol-gel transition temperature (A) Digital image of
11 poloxamer formulation in sol form (left), and in gel form (right), reproduced with permission from
12 [20]; (B) DSC thermogram of poloxamer 407 (20% w/w) showing sol-gel transition temperature at
13 24°C, reproduced with permission from [28]; (C) Viscosity, storage and loss moduli of poloxamer
14 formulation showing sol-gel transition temperature at 34°C reproduced from [29] under CC BY
15 license.

16 **Figure 3** Device for determination of mucoadhesive force of the gel formulation to the corneal
17 membrane according to the description of Shastri *et al* [47], figure was developed by the authors.

18 **Figure 4** (A) Rabbit eye after intravitreal injection of poloxamer 127 formulation relative to control
19 eye reproduced from [59] under CC BY license; (B) Anatomy of human eye; (C) Optical coherence
20 tomography showing the use of 500 µm-long microneedles for intrascleral injection of poloxamers
21 solution, reproduced with permission from [36].

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