International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 13, Issue 1, 2021

Review Article

A REVIEW ON POLOXAMER AND HYDROXY PROPYL METHYL CELLULOSE COMBINATION AS THERMORESPONSIVE POLYMERS IN NOVEL OPHTHALMIC IN SITU GEL FORMULATION AND THEIR CHARACTERIZATION

INSAN SUNAN KURNIAWANSYAH[1*], TAOFIK RUSDIANA[1], IYAN SOPYAN[2], ANAS SUBARNAS[3]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, West Java, Indonesia, [2]PUSDI Drug Delivery and Drug Disposition Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, West Java, Indonesia, [3]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, West Java, Indonesia
Email: insan.sunan.kurniawansyah@unpad.ac.id

Received: 11 Sep 2020, Revised and Accepted: 17 Oct 2020

ABSTRACT

Poor bioavailability is one of the most significant problems in the delivery of the ocular drug system. Ophthalmic ointments, solutions and suspensions are the most frequently used dosage forms to treat ocular disease, and their effectiveness as a drug are compromised by several limitations that lead to poor ocular bioavailability. *In situ* gel is one of the most promising strategy and solutions to improve the ocular bioavailability of drugs. The purpose of this review is to discuss the formulation and characterization of *in situ* gel. This review is written based on the data or information obtained by using several search engines and several scientific journals, focused on Poloxamer 407 and Hydroxy Propyl Methyl Cellulose (HPMC) bases combination.

Active ingredients to treat ocular disease such as Ciprofloxacin, Fluconazole and Ofloxacin can be formulated with the combination of Poloxamer 407 as polymer gelling agent and HPMC as viscosity enhancer to produce good quality *in situ* gel dosage forms. The *in situ* gel dosage forms can be a promising alternate solution for the ophthalmic delivery system.

Keywords: *In situ* gel, Ocular disease, Bioavailability, Poloxamer 407, HPMC, Ciprofloxacin, Fluconazole, Ofloxacin

DOI: http://dx.doi.org/10.22159/ijap.2021v13i1.39697. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Topical application of drugs is the most common route of administration for the treatment of many eye conditions. The eyes is special because of the structural and functional aspect of this organ makes it highly impervious to foreign substances and material. Protective mechanisms of the eye such as blinking, baseline and reflex lachrymation and drainage remove foreign ingredients including drugs from the eye's surface. Because of it, targeting of drugs to eye tissues is one of the hardest challenge in drug delivery [1, 2].

Conventional and frequently used ophthalmic dosage forms such as solutions and suspensions have several drawbacks, for example-rapid precorneal elimination of drugs because of the nasolacrimal drainage, the need for frequent application and pulse release. Other dosage forms, which is ophthalmic ointments, provide prolonged contact with the eye surface. But it may trigger some side effects such as foreign body sensation, blurred vision and inconvenience to the patient [2, 3].

Physiological constraints imposed by the eye's protective mechanism lead to low absorption of drugs and short duration of therapeutic effect. Drug absorption is also influenced by the chemical nature of the drugs, since the permeability of cornea depends on molecular size and hydrophobicity of drugs. In order to increase drug effectiveness, a dosage form that increases the contact time of the drug in the eye should be selected. This can lead into increased bioavailability, reduced systemic absorption and reduce the need for frequent drug administration, leading to the improvement of patient compliance. One of the solution to increase precorneal residence time is by increasing the viscosity of drug [4].

This condition can be achieved by gelling systems or in this case *in situ* gel dosage form [5]. The purpose of this journal review are to discuss the formulation and characterization of *in situ* gel drug made using a combination of poloxamer 407 and HPMC bases.

Methods

The source of data for this journal review about *in situ* gel formulation and characterization are summarized and cited from several international journals, obtained from the internet using internet browser software and search engine. 50 international journals are used as a reference, obtained from various sources. The keywords used include *in situ* gel, formulation, characterization, poloxamer, Hydroxy Propyl Methyl Cellulose/HPMC.

DISCUSSION

In situ gelling systems can be defined as viscous liquids, which undergo a phase transition from solution to gel when applied to the human body [1]. *In situ*-forming hydrogels can also be described as liquid form upon instillation and undergo phase transition in the ocular cul-de-sac to form a viscoelastic gel and this provides a response to environmental changes [5]. The triggers to phase change are either temperature, pH or ionic strength.

In the temperature-based method, gelling of the solution is triggered by change in the temperature. Sustained drug delivery can be achieved by the use of a polymer that changes from solution to gel at the eyes temperature. But the disadvantage of this method is characterized by very high polymer concentration. The examples of temperature-based in situ gel is methylcellulose and smart hydrogels. In pH based method, gelling of the solution is triggered by a change in the pH. CAP latex cross linked polyacrylic acid and its derivatives such as carbomers are used. They are low viscosity polymeric dispersion in water, which undergoes spontaneous coagulation and gelation after instillation in the conjunctival cul-de-sac. In ionic strength-based method, of the solution, instilled is triggered by change in the ionic strength [6].

Gels that undergo sol-gel phase transition caused by a temperature change are called thermoresponsive *in situ* gels. Such gels have been extensively investigated as temperature-responsive materials for invertible thermoresponsive gelation under certain temperatures and concentrations [7].

The efficacy of ophthalmic hydrogels is mostly based on an increase of ocular residence time by enhancing the viscosity and mucoadhesive properties. Since resulted swollen hydrogel is aqueous-based, it is very comfortable in the patient's eye. Because of this, *in situ* gels are preferred since they are conveniently dropped in the eye as a solution, where undergo transition into a gel [8].

One of the journals claims that among all *in situ* gel-forming systems, activation by change in ionic strength is most effective. The advantage is based on the fact that fluctuations in pH and temperature, which could cause changes in the gelation process, are not associated with the ion-activated system. These fluctuations in pH could cause ocular irritation, and storage conditions could lead to changes in temperature [9].

There are several mechanisms of *in situ* gels based on its physical mechanism: swelling, diffusion and formation based on the chemical reaction. In the swelling mechanism, *in situ* formation may also occur when a material absorbs water from the surrounding environment and expand to the desired space. One such substance is myverol (glycerol mono-oleate), which is polar lipid that swells in water to form lyotropic liquid crystalline phase structures [10].

In the diffusion mechanism, it involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. N-methyl pyrrolidone (NMP) has been shown to be useful solvent for such system. And in the chemical reaction mechanism, chemical reactions that results *in situ* gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes, and photo-initiated processes [11-13].

According to this journal written by Chand *et al.*, The polymers used in *in situ* gel should be nontoxic and non-absorbable from the gastrointestinal tract. It should adhere quickly to moist tissue and should possess some site-specificity, be a non-irritant to the mucous membranes, and possess a wide margin of safety both locally and systemically. Another important thing, the cost of the polymer should be not too high so that prepared dosage form remains competitive [14].

Another journal written by Saini et al., describe the ideal characteristics of polymers for the preparation of in situ ophthalmic gel. It should be biocompatible, capable of adhering to mucus membrane, have pseudoplastic behavior, has good tolerance and optical clarity, influences the tear behavior and the polymer should be capable of decreasing the viscosity with increasing shear rate [15].

Laddha and Mahajan also describe three ideal properties for *in situ* formulations: physical state, phase transition and strength of gel [16]. First, the formulation should be a free-flowing liquid which allows ease of administration with reproducible dose delivery. Second, upon instillation, it should undergoes sol-to-gel formation by phase transition [17]. And the third, formed gel should be strong enough to withstand the shear force in the cul-de-sac, which prolongs the residence time of the drug [18].

Poloxamer and HPMC are two of the most widely used excipients in the formulation of *in situ* gel, mainly for the temperature-based type. Temperature-sensitive systems are the most commonly studied class of stimuli-responsive polymer systems for ocular targeting [19]. The use of a biomaterial that undermines the transition process from sol to gel form is triggered by a change in temperature is an attractive way to achieve *in situ* formation [20]. Physiologic temperature where there is no need of an external source of heat other than that of the body for gelation is the ideal phase transition temperature for this type of systems. Temperature-sensitive *in situ* gels can be classified into negatively thermosensitive, positively thermo-sensitive, and thermally reversible gels [21]. Poloxamer (or Pluronic) is the most commonly used polymers in preparations of thermosensitive *in situ* gel. Another widely used polymers are xyloglucan, chitosan and naturally occurring cellulose derivative. HPMC and MC belongs to the cellulose derivative group of the polymer [22]. Its also employed as another basic formulation component of *in situ* gel formulation. It functions as a viscosity enhancer in order to achieve the desired consistency so as to facilitate sustained drug release [23].

Poloxamer are a water-soluble tri-block copolymer that consisted of two polyethylene oxide and polypropylene oxide core in an ABA configuration. Poloxamer commercially, also known as pluronic and has good thermal setting property and increased drug residence time. It is used as a gelling agent and solubilizing agent. Poloxamer gives colorless, transparent gel. The mechanism of poloxamer is as follows: At room temperature (25 °C), it behaves as a viscous liquid and is transformed to transparent gel when temperature increases (37 °C). At low temperature, it forms a small micellar subunit in solution and increases in temperature results increase in viscosity, leads to swelling to form a large micellar cross-linked network [24, 25].

Poloxamers, commercially available and sold as Pluronic®, are triblock copolymers composed of polyethylene oxide (PEO) units and polypropylene oxide (PPO) units (PEO/PPO/PEO). It exhibits reverse thermal gelation under a certain temperature and concentration. Pluronic F68 (PF68), which is an analog of Pluronic F127 (PF127), was added to PF127 solution to increase its gelation temperature [26].

Poloxamer 407 (PF-127) is a nonionic surfactant composed of poly (ethylene oxide)-b(poly(propylene oxide)-b-poly(ethylene oxide) (PEO-PPOPEO) that shows amphiphilic behavior because of hydrophobic propylene oxide domains and hydrophilic ethylene oxide domains [27]. Pluronic F127 exhibits sol to gel transition at 37 °C when used at a higher concentration of (25%-30%) (w/v). By using different series of poloxamers, cross-linking agents, by changing pH and ionic strength gelation, the temperature can be adjusted within the range of 33-36 °C [28].

HPMC is a semisynthetic, inert, viscoelastic polymer which is non-ionic nontoxic, a good carrier for application of pharmaceutical drug which shows high swelling capacity [29, 30]. Methylcellulose solutions transform into opaque gel between 40-50 °C, whereas HPMC shows a phase transition between 75-90 °C. These phase transition temperatures can be lowered by chemical or physica modifications [31]. The mechanism of HPMC gellation process is as follows: Gelation of cellulose solutions is caused by the hydrophobic interactions between molecules containing methoxy substitution. At low temperatures, the macromolecules are hydrated and polymer-polymer interaction is minimal. As the temperature is raised, the polymers will gradually lose their water of hydration. This condition is reflected by a decline in relative viscosity [32]. For the main discussion, we will compare three journals about Poloxamer 407 and HPMC combination as reference.

A. Controlled ocular drug delivery of ofloxacin using temperature modulated *in situ* gelling systems. Authors: Upendra Nagaich, Nidhi Jain, Divya Kumar and Neha Gulati. Published in Journal of the Scientific Society, Vol 40/Issue 2/May-August 2013 [32].

B. *In situ* Gelling Ophthalmic Formulations for Sustained Release and Enhanced Ocular Delivery of Fluconazole. Authors: Nabil Abdullah Ali Khatera, Osama A. Soliman and Elham A. Mohamed. Published in IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) Volume 11, Issue 2 Ver. I (Mar.-Apr.2016), PP 43-51 [33].

C. Ophthalmic *In situ* Sustained Gel of Ciprofloxacin, Preparation and Evaluation Study. Authors: Fadia Yassir Al-Bazzaz and Myasar Al-Kotaji. Published in International Journal of Applied Pharmaceutics Vol 10, Issue 4, 2018 [1].

Formulation

Formulation of *in situ* gel from journal A

Table 1: Formula of developed in situ gel systems

Formulation	FI	F2	F3	F4	F5
Ofloxacin (% w/v)	0.3	0.3	0.3	0.3	0.3
Pluronic F 127 (% w/v)	16	18	20	22	24
HPMC 50 cps (% w/v)	1.5	1.5	1.5	1.5	1.5
NaCl (% w/v)	0.51	0.51	0.51	0.51	0.51
Benzalkonium chloride (% w/v)	0.01	0.01	0.01	0.01	0.01
Disodium hydrogen phosphate	1.125	1.125	1.125	1.125	1.125
Distilled water (ml)	100	100	100	100	100

Formulation of *in situ* gel from journal B

Table 2: Composition of fluconazole selected *in situ* gel formulations

Ingredients	Formulation code			
	Temperature triggered in situ gelling system		Ion activated in situ gelling system	
	FI	F2	F3	F4
Fluconazole	0.3	0.3	0.3	0.3
P407	15	15	-	-
HPMC	2	3	2	3
ALG	-	-	0.4	0.3
BKC	0.01	0.01	0.01	0.01
Water q. s.	100	100	100	100

Formulation of *in situ* gel from journal C

Table 3: Composition of ophthalmic in situ gels of ciprofloxacin hydrochloride % (w/w)

Formula	Ciprofloxacin HC1	P407	HPMC	Benzalkonium chloride	Sodium chloride equivalent to
F1	0.3	13	0.2	0.02	0.9
F2	0.3	13	0.4	0.02	0.9
F3	0.3	15	0.6	0.02	0.9
F4	0.3	16.25	0.2	0.02	0.9
F5	0.3	16.25	0.4	0.02	0.9
F6	0.3	16.25	0.6	0.02	0.9
F7	0.3	17.5	0.2	0.02	0.9
F8	0.3	17.5	0.4	0.02	0.9
F9	0.3	17.5	0.6	0.02	0.9
Fl0	0.3	20	0.4	0.02	0.9
F11	0.3	16.25	0	0.02	0.9
F12	0.3	17.5	0	0.02	0.9

The differences between all three formulations from each journal is the active ingredients used. Ofloxacin for the first, Fluconazole for the second and Ciprofloxacin for the third. Ofloxacin and Ciprofloxacin acts as an antibiotic, whereas Fluconazole on the other hand acts as an antifungal. The concentration of the excipients such as Poloxamer 407, HPMC, and Benzalkonium Chloride as preservative ingredients is different too. Another differences between the three of them is the amount of formulations variant. The third journals use 12 formula, higher than the other two journals (five and four formula for the first and the second journal). Because of this, the result from Journal C is better from Journal A and B.

Preparation methods

a. Journal A (Controlled ocular drug delivery of ofloxacin using temperature modulated *in situ* gelling system)

Aqueous solutions of Pluronic F127 and HPMC were prepared by dispersing them in distilled water with constant stirring. Ofloxacin was dissolved in glacial acetic acid and added to HPMC solution. HPMC drug solution was then poured into Pluronic solution with constant agitation and was allowed to stand at 4 °C for 24 h to make a clear solution [32].

b. Journal B (*In situ* gelling ophthalmic formulations for sustained release and enhanced ocular delivery of fluconazole)

Different *in situ* gelling preparations of FLZ (Fluconazole) were prepared under aseptic conditions to ensure the sterility of the final products. Based on the gelling capacity results, optimal concentration(s) of each polymer (P407, ALG, and CP934) with HPMC were determined. The required amount of each polymer was dispersed in 45 ml phosphate buffer saline (PBS) containing 0.01% benzalkonium chloride (BKC) as a preservative and stirred till complete dissolution. FLZ (0.3% w/v) was dissolved in 45 ml PBS and HPMC was added. The two resultant solutions were mixed using a magnetic stirrer. The volume was adjusted to 100 ml and transferred to a dry, clean, and sterile glass bottle.

In situ gel of P407 was prepared by following the same method except that the polymer was added to previously cooled PBS to 4 °C. For plain formulations used for assessment of gelling capacity were prepared at the same method except that FLZ was not added [33].

c. Journal C (Ophthalmic *in situ* sustained gel of ciprofloxacin, preparation and evaluation study)

Twelve different formulas of ciprofloxacin HCl *in situ* gel were prepared by using different concentrations of (P407) with different concentrations of HPMC.

An *in situ* gel of ciprofloxacin was prepared by using the cold method [10, 34]. The required amount of (P407) was added to acetate buffer (pH 4.5) (at 4 °C) with gentle mixing and then allowed to hydrate overnight at the same temperature. The required amount of viscosity enhancing agent, HPMC, was dissolved in hot acetate buffer and cooled to 4 °C and then added to polymer solutions. Ciprofloxacin HCl was dissolved in the required amount of acetate buffer and then sodium chloride and benzalkonium chloride were added to the solution. After that, the drug solution was added to the polymer solution and mixed.

The samples were then transferred to vials and stored in a refrigerator overnight. Finally, the vials were sterilized by autoclave at 121 °C at 15 psi for 20 min [1, 35].

The differences between the three preparation methods above is the sterilization process. Method from Journal B and C explains the aseptic and sterilization process to ensure that the product is in a sterile condition.

Characterization

Characterization and evaluation of *in situ* gel from the three journal is similar.

a. Visual appearance and clarity

The appearance of the prepared *in situ* gel systems was determined visually. Clarity of the *in situ* gel was observed against a white and black background for presence of any particulate matter [1, 36, 37].

b. Drug content

In situ gel forming systems were also characterized for the drug content. 1 ml of the preparation was left for 24 h in simulated artificial tear fluid (pH 7.4) and the sample was analyzed in ultraviolet spectrophotometer (UV 1601, Shimadzu, Japan) at 288 nm [1, 37, 38].

c. Viscosity and pH

The viscosity was measured using a Brookfield viscometer (LV-DVE model, Brookfield, USA). pH of the formulation was measured using a digital pH meter [1, 37-39].

d. Gelling capacity

The gelling capacity was determined by placing one drop of the formulation in a vial containing 2 ml of freshly prepared artificial tear fluid and visually assessing time for gelation and the time taken for the gel to redissolve. The composition of artificial tear fluid used was NaCl 0.670 g, sodium bicarbonate 0.200 g, calcium chloride $2H_2O$ 0.008 g, in 100.0 ml of purified water [1, 40].

e. Ocular irritancy test

Draize irritancy test was performed on male albino rabbit (or female rabbit in Journal C) in order to demonstrate the safety potential of prepared formulation in animal model. A total of 100 µl of the optimized batch formula was placed in the lower cul-de-sac and was observed at various time intervals. Rabbits were visually examined for any redness, swelling or excessive tear production [1, 41].

f. *In vitro* studies

Dissolution studies of samples were performed using Franz diffusion apparatus and phosphate buffer (pH 7.4) as a dissolution medium. Phosphate buffer with pH 7.4 will simulate the lachrymal fluid. The temperature was maintained at 37+0.5 °C with the speed of rotation maintained at 100 rpm. The samples were withdrawn at various time intervals and analysed spectrophotometrically for the drug content [1, 42-46].

g. *In vivo* studies

In vivo therapeutic efficacy of developed optimized formulation and marketed eye drop formulation was investigated by using normotensive New Zeland albino rabbits. Intra ocular pressure of normotensive rabbits decreased within 1 h administration of single dose of developed in situ gel system and showed 10.86±4.33 % reduction which continued upto 8 h with 31.22±3.65 % reduction ($p<0.01$). It is worthy to note that marketed eye drop formulation immediately reduced intraocular pressure with 18.22±4.42 % up to 2 to 3 h. From the *in vivo* study, it was concluded that the after administration of developed in situ gel in the rabbit eye, it turned out in gel form. During gel formulation, formulation got converted into the gel and thus drug release become slowly. Developed formulation confirmed gelling ability of poloxamer-407 at physiological conditions where as HPMC K15M enhances viscosity and sustained release profile of developed formulation. It was also observed that initial reduction in intraocular pressure was very rapid, this may be due to slow conversion of solution to gel formulation, but the reduction in intraocular pressure became slow in latter period after gel formation. It was observed after administration of marketed eye drop formulation, the rapid reduction in intraocular pressure upto 3 h, but due to liquid nature of formulation it had no ability to retain in cul de sac region. The results showed that after 3 h % decrease in IOP again decrease in case of marketed formulation due to its liquid nature [47, 48-50].

Evaluation result from all three journals and formulations shows that the developed formulation of *in situ* gel using combination of Poloxamer 407 (Pluronic F127) as gelling agent and HPMC as viscosity modifying agent is succesfully formulated. The optimized formula from each of the three journals represent a promising alternative solution to the conventional ophthalmic dosage forms because of its ability to enhance bioavailability.

CONCLUSION

Ophthalmic *in situ* gel is able to change into gel when applied to the eye. An *in situ* gel drug delivery system provides several benefits, such as prolonged pharmacological duration of action, simpler production techniques, and low cost of manufacturing as compared to conventional drug delivery systems. Poloxamer 407 is a well-known stimuli-responsive polymer type with thermoresponsive behaviour. It is commonly used as an eye drug delivery system as it could prolong drug release for eye tissue. The addition of hydroxypropyl methyl cellulose (HPMC) can reduce the concentration of poloxamer 407 needed to form *in situ* gel gelation. Active ingredients to treat ocular disease such as Ciprofloxacin, Fluconazole and Ofloxacin can be formulated with the combination of Poloxamer 407 as polymer gelling agent and HPMC as viscosity enhancer to produce a good quality *in situ* gel dosage forms. Compared to the conventional ophthalmic dosage, the *in situ* gel dosage forms can be a promising alternate solution for ophthalmic delivery system.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

REFERENCES

1. Al-Bazzaz, Al-Kotaji. Ophthalmic in-situ sustained gel of ciprofloxacin, preparation and evaluation study. Int J Appl Pharm 2018;10:153-61.
2. Wu Y, Liu Y, Li X, Kebebe D, Zhang B, Ren J, *et al.* Research progress of in-situ gelling ophthalmic drug delivery system. Asian J Pharm Sci 2019;14:1-15.
3. Fathalla MA, Vangala ZA, Longman M, Khaled KA, Hussein AK, ElGarhy OH, Alany RG. Poloxamer-based thermoresponsive ketorolac tromethamine in situ gel preparations: design, characterisation, toxicity and transcorneal permeation studies. Eur J Pharm Biopharm 2017;114:119-34.
4. Preethi GB, Narendra E. Formulation and evaluation of in situ mucoadhesive ophthalmic hydrogel for sustained delivery of pefloxacin mesylate. Int J Pharm Pharm Sci 2015;7:345-50.
5. Anuja TK, Rahul LJ, Pradnya BS, Satwashila SK. Design and evaluation of modified chitosan based in situ gel for ocular drug delivery. Int J Pharm Pharm Sci 2017;9:87-91.
6. Agarwal K, Mehta N, Namdev A, Gupta A. In-situ gel formation for ocular drug delivery system an overview. Asian J Biomed Pharm Sci 2011;1:1-7.
7. Lou J, Hu W, Tian R, Zhang H, Jia Y, Zhang J, *et al*. Optimization and evaluation of a thermoresponsive ophthalmic *in situ* gel containing curcumin-loaded albumin nanoparticles. Int J Nanomed 2014;9:2517-25.
8. Rajoria G, Gupta A. In-situ gelling system: a novel approach for ocular drug delivery. Am J PharmTech Res 2012;2:24-53.
9. Kotreka UK, Davis VL, Adeyeye MC. Development of topical ophthalmic *In situ* gel forming estradiol delivery system intended for the prevention of age-related cataracts. Plos One 2017;12:1-19.
10. Lin HR, Sung KC. Carbopol/Pluronic phase change solutions for ophthalmic drug delivery. J Controlled Release 2000;69:379-88.
11. Parekh HB, Rishad J, Jivani NP, Patel LD, Makwana A, Sameja K. Novel *in situ* polymeric drug delivery system: a review. J Drug Delivery Ther 2012;2:136-45.
12. Nirmal HB, Bakliwal SR, Pawar SP. *In situ* gel: new trends in controlled and sustained drug delivery system. Int J Pharm Tech Res 2010;2:1398-408.
13. Nerkar TS, Gujarathi NA, Bhushan R, Bakliwal SR, Pawar SP. *In situ* gel: novel approach in sustained and controlled drug delivery system. Pharma Sci Monitor 2013;4:1-18.
14. Chand P, Pratibha, Gnnarajan G, Kothiyal. *In situ* gel: a review. Indian J Pharm Biol Res 2016;4:11-9.
15. Saini R, Saini S, Singh G, Banerjee A. *In situ* gels-a new trends in ophthalmic drug delivery systems. Int J Pharm Sci Res 2015;6:886-90.
16. Laddha UD, Mahajan HS. An insight to ocular *in situ* gelling systems. Int J Adv Pharm 2017;6:31-40.
17. El Laithy H, Nesseem D, Shoukry M. Evaluation of two *in situ* gelling systems for ocular delivery of Moxifloxacin: *in vitro* and *in vivo* studies. J Chem Pharm Res 2011;3:66-79.
18. Rathore KS. *In situ* gelling ophthalmic drug delivery system: an overview. Int J Pharm Sci Res 2010;2:30-4.

19. Cao Y, Zhang C, Shen W. Poly (N-isopropylacrylamide)-chitosan as thermosensitive *in situ* gel-forming system for ocular drug delivery. J Controlled Release 2007;120:186-94.
20. Kute PR, Gondkar SB, Saudagar RB. Ophthalmic in-situ gel: an overview. World J Pharm Pharm Sci 2015;4:549-68.
21. Israr S, Nesalin J, Mani TT. A novel approach of ophthalmic drug delivery: *in situ* gel. J Pharm Res 2015;4:121-4.
22. Gowda DV, Tanuja D, Khan MS, Desai J, Shivakumar HG. Formulation and evaluation of in-situ gel of diltiazem hydrochloride for nasal delivery. Pharm Lett 2011;3:371-81.
23. Makwana SB, Patel VA, Parmar SJ. Development and characterization of in-situ gel for ophthalmic formulation containing ciprofloxacin hydrochloride. Results Pharm Sci 2016;6:1-6.
24. Aishwarya JJ, Sheetal BG, Ravindra BS. A review on nasal drug delivery system. World J Pharm Pharm Sci 2014;3:231-54.
25. Prajapati N, Goyal A. A review: thermoreversible mucoadhesive in-situ gel. Int J Innovative Drug Discovery 2013;3:67-84.
26. Pawar P, Kashyap H, Malhotra S, Rakesh S. Hp-β-CD-voriconazole *in situ* gelling system for ocular drug delivery: *in vitro*, stability, and antifungal activities assessment. Biomed Research International 2013:1-9. DOI:10.1155/2013/341218
27. Choi HG, Jung JH, Ryu JM, Yoon SJ, Oh SYK, Kim CK. Development of *in situ*-gelling and mucoadhesive acetaminophen liquid suppository. Int J Pharm 1998;165:33-44.
28. Geethalakshmi A, Karki R, Jha SK, Venkatesh DP, Nikunj B. Sustained ocular delivery of brimonidine tartrate using ion activated *in situ* gelling system. Curr Drug Delivery 2012;9:197-204.
29. Wee S, Gombotz WR. Protein release from alginate matrices. Adv Drug Delivery Rev 1998;31:267-85.
30. Champalal KD, Sushilkumar P. Current status of ophthalmic *in situ* forming hydrogel. Int J Pharm Bio Sci 2012;3:372-88.
31. Nanjawade. *In situ* forming hydrogels for sustained ophthalmic drug delivery. J Controlled Release 2007;122:119-34.
32. Nagaich U, Jain N, Kumar D, Gulati N. Controlled ocular drug delivery of ofloxacin using temperature modulated *in situ* gelling system. J Sci Soc 2013;40:90-4.
33. Ali Khatera NA, Soliman OA, Mohamed EA. *In situ* gelling ophthalmic formulations for sustained release and enhanced ocular delivery of fluconazole. J Pharm Biol Sci 2016;11:43-51.
34. Kumar D, Jain N, Gulati N, Nagaich U. Nanoparticles laden in situ gelling system for ocular drug targeting. J Adv Pharm Technol Res 2013;4:9-17.
35. Jethava JK, Jethava GK. Design, formulation, and evaluation of novel sustain release bioadhesive in-situ gelling ocular inserts of ketorolac tromethamine. Int J Pharm Investig 2014;4:226-32.
36. Kurniawansyah IS, Rusdiana T, Wahab HA, Subarnas A. *In situ* ophthalmic gel with ion activated system. Int J Appl Pharm 2019;11:15-8.
37. Jain D, Kumar V, Singh S, Mullertz A, Bar Shalom D. Newer trends in in situ gelling systems for controlled ocular drug delivery. J Anal Pharm Res 2016;2:22.
38. Deka M, Ahmed AB, Chakraborty J. Development, evaluation and characteristics of ophthalmic *in situ* gel system: a review. Int J Curr Pharm Res 2019;11:47-53.
39. Majeed A, Khan NA. Ocular *in situ* gel: an overview. J Drug Delivery Ther 2019;9:337-47.
40. Madan JR, Adokar BR, Dua K. Development and evaluation of *in situ* gel of pregabalin. Int J Pharm Investig 2015;5:226-33.
41. Patel N, Thakkar V, Metalia V, Baldaniya L, Gandhi T, Gohel M. Formulation and development of ophthalmic *in situ* gel for the treatment ocular inflammation and infection using application of quality by design concept. Drug Dev Ind Pharm 2016;42:1406-23.
42. Kurniawansyah IS, Sopyan I, Wardhana YW, Gunasekaran M. Formulation and evaluation of chlorampenicol hydrogel opthalmic preparation. J Young Pharm 2018;10:73-8.
43. Kadam S, Kondawar M, Kamble K. Formulation and evaluation of in situ gelling system of ketrolac tromethamine for ophthalmic drug delivery. Int J Pharm Ther 2010;1:64-71.
44. Shashank NN, Srinivasa U. Design and evaluation of ion activated *in situ* ophthalmic gel of moxifloxacin hydrochloride and ketorolac tromethamine combination using carboxy methylated tamarind kernel powder. Saudi J Med Pharm Sci 2017;3:1-8.
45. Verma L, Sakir M, Singh N, Gilhotra MN, Mehan S. Development of phase change solutions for ophthalmic drug delivery based on ion activated and pH induced polymers. Int J Pharma Professional's Res 2010;1:127-34.
46. Sundari PJP, Maddela R, Srinivas P. Formulation and evaluation of thermosenstive ocular *in situ* gels for sustained release of balofloxacin. World J Pharm Pharm Sci 2015;4:835-45.
47. Barse RK, Tagalpallewar AA, Kokare CR, Sharma JP, Sharma PK. Formulation and ex vivo-*in vivo* evaluation of pH-triggered brimonidine tartrate *in situ* gel for the glaucoma treatment using application of 3^2 factorial design. Drug Dev Ind Pharm 2017;44:800-7.
48. Sun J, Zhou Z. A novel ocular delivery of brinzolamide based on gellan gum: *in vitro* and *in vivo* evaluation. Drug Des Dev Ther 2018;12:383–9.
49. Kashikar VS, Gonjari ID. In situ gelling systems of ofloxacin: Comparative performance of *in vivo* precorneal drainage and pharmacokinetic study. Asian J Pharm 2013;7:15-20.
50. Balasubramaniam J, Kant S, Pandit JK. *In vitro* and *in vivo* evaluation of the Gelrite® gellan gum-based ocular delivery system for indomethacin. Acta Pharm 2003;53:251–61.