ejpmr, 2020,7(6), 941-949

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVOLUTION AND ASSESSMENT OF OPHTHALMIC IN-SITU GEL CONTAINING AZITHROMYCIN

Rashmi B.*, Somashekhar C. N. and D. V. Gowda

Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagar, Maddur Taluk, Mandya District, Karnataka, India. 571422.

***Corresponding Author: Rashmi B.**

Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagar, Maddur Taluk, Mandya District, Karnataka, India. 571422.

Article Received on 21/04/2020 **Article Revised on 11/05/2020** **Article Accepted on 01/06/2020**

ABSTRACT

The aim of present work is to formulate and evaluate the thermo sensitive drug delivery system of azithromycin for the treatment of conjunctivitis in the form of *in situ* gel to overcome problem associated with the conventional eye drops such as rapid precorneal elimination, poor bioavailability and nasolacrimal drainage. The thermo reversible ophthalmic in situ gel were prepared by cold method using pluronic f 127 as thermosensitve polymer, HPMC K100LV as viscosity enhancing agent, carbopol as gelling agent all the three polymers are used in different concentration in different proportions, macrolide antibiotic drug azithromycin and benzalkonium chloride is used as preservative. Prepared formulation were subject for several evaluation. The developed thermo reversible *in situ* gel shows the sustained drug releases profile and was found to be stable, nonirritant and also passes the sterility test.

KEYWORDS: Azithromycin, thermo sensitive drug delivery system, pluronic f127, HPMC K15M, carbopol, *In-situ* gel.

INTRODUCTION

The eye is contemplated as the opening to the human soul and it is one of the difficult and unique part of human body.[1] Due to its drug temperament characteristic eye is considered as the one of the most interesting sensory organs and this organ can easily change the light to the electrical signal which is elucidated by the brain. Generally in the ophthalmic therapy more than the other routes topical route of drug administration is considered as preferred way because of its convenience and safety.[2],[3]

In the last few decennium, sufficiently greater attentions has been concentrated on advancement of controlled and sustained drug delivery system[4] in order to develop the stable sustained release ophthalmic in situ gel to overcome the problem related with the conventional ophthalmic dosage form such as eye drops which are easy to instill but show some inherent draw back such as nasolacrimal drainage, poor bioavailability and quick pre corneal expulsion of drug, this can result in the undesired systemic side effect.[5]

Applying In situ gelling ophthalmic drug delivery system it is easy to overcome the difficulties that are related with the conventional dosage form. This *in situ* gels were prepared by using polymers were the liquid state of the formulation is converted into gel form at the site of application that means these exhibit the sol to gel transition because of the specific physiological changes in environment such as temperature, pH, solvent exchange, ion cross linking, and ultraviolet irradiation. Due to sol to gel transition of the solution this may remain in the eye for the longer period their by reduce the frequency of dosing and concentration, improve the biocompatibility, patient complaints and enhances the ocular bioavailability by enhancing the pre corneal residence time.[6],[7]

The bacterial conjunctivitis characterized as pink eye, were the inflammation of eye caused due to the enlargement of blood vessels, which resulting in red and blood shot appearance in the eye. Several antibiotics are used for the treatment of conjunctivitis like Azithromycin, Erythromycin, Gentamycin, Ciprofloxacin etc. The drug used is Azithromycin which is macrolide antibiotic used in the treatment of several bacterial infections such as mid ear infection, strep throat, pneumonia and other intestinal infection etc. Mainly it is used in treatment of trachoma caused by *Chlamydia trachomatis*, a gram negative bacteria. It is suitable to treat the ocular infections such as conjunctivitis and others caused by sensitive pathogens. Topical ocular delivery is found to be useful in treating conjunctivitis whose dose is 0.5 %w/v.[8], [9]

MATERIALS AND METHODS

Azithromycin was a gift sample from Avyukt pharmaceutical Ltd, Bengaluru, Pluronicf127were gifted from SIGMA life science, carbopol 934 were gifted from Rolex chemicals, Mumbai, HPMC K100LV from Shreeji chemicals, Mumbai, sodium chloride and disodium EDTA from nice chemical Pvt Ltd, Kerala, benzalkonium chloride from s d fine-Chem limited, Mumbai. And all other chemicals from laboratory grade.

Fourier transforms infra-red spectroscopy (FT-IR) analysis

In this method FT-IR spectra of the pure dug and physical mixture were carried out using a Fourier transform infrared spectrophotometer. The scanning was accomplish by using Shimadzu FT-IR-8400s spectrophotometer (Europe). Over a wave number range of 400 to 4000 cm^{-1} at 4 cm^{-1} spectra was scanned .Then sample was scattered in the KBr and flatten into pellets by applying pressure. For recoding the IR spectra the obtained pellets were kept in path of light.

DSC (Differential scanning calorimeter)

The DSC examination of pure drug and polymer combination were accomplish by utilizing a Shimadzu DSC-60(PerkinElmer, USA) calorimeter to assess any feasible drug polymer interaction. The scanning was carried out at a rate of 5.00 ºC min^{-1} from 10 °C to 300 °C temperature range under nitrogen flow of 25ml min^{-1} [10].

X-ray diffractrometer (XRD)

X-ray diffraction design of pure drug Azithromycin and its physical mixture were documented by utilizing (PROTO AXRD, bench top system, Canada) X- ray diffractrometer with a copper target, voltage 40.00 Kv, current 30.00 MA at a scanning speed of 0.30°C/min.[11]

Proton nuclear magnetic resonance (^{1}HNMR)

^{1}HNMR was done for pure drug Azithromycin and its physical mixture were recorded using VNMRS-400 "Agilent-NMR".[12]

Preparation of thermo reversible ophthalmic in situ gel[5],[13]

Thermo reversible in situ gel of Azithromycin was prepared by cold method using mechanical stirrer. In these formulation three different polymers are used in different concentration and ratios such as pluronic f127 (10-20%), carbopol (0.1-0.5%) and HPMC K100LV (1.5%). Accurately weighed quantity of pluronic f127 and bio adhesive polymers and benzalkonium chloride were dissolved in phosphate buffer of pH 6.0. To the above solution needed amount of Azithromycin was added and mixed well under continuous stirring till constant solution were obtained then to this solution add disodium EDTA and sodium chloride and then by using buffer pH 6.0 (phosphate buffer) made up the final volume. Then the prepared solutions are stored at 4°C for overnight refrigeration which resulted into clear solution. The developed formulations were filled in the glass vials, closed with butyl rubber closure. Then the prepared formulations were subjected for evaluation tests.

Table 1: Composition of temperature triggering in situ gel of Azithromycin.

Ingredients in gm.	Formulations				
	F1	F2	F3	F4	F5
Azithromycin	0.5	0.5	0.5	0.5	0.5
Pluronic f127	10	10	10	10	10
HPMC K100LV	1.5	1.5	1.5	1.5	1.5
Carbopol 934	0.1	0.2	0.3	0.4	0.5
Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02
Disodium EDTA	0.01	0.01	0.01	0.01	0.01
sodium chloride	0.1	0.1	0.1	0.1	0.1
Phosphate buffer pH 6.0 q.s (ml)	100	100	100	100	100

Evaluation of in situ gel

Appearance and clarity

In this method the fluorescence light was used against the white and black back ground to check the appearance and clarity of the formulation. The formulation was examined for any particulate matter and turbidity.[14]

pH of gel

pH was checked by using pH meter. The preparation was taken in beaker and 1ml NaOH added drop wise with constant stirring. Then this mixture was analysed.[15]

Sol-Gel transition temperature

The solution to gel phase transformation temperature may be explained as the temperature at which the phase transformation of the solution to the gel takes place when it is placed in the sample tube at the particular temperature and then heated at a certain rate. When there was scarcity of movement of the meniscus upon tilting of the tube, gel formation was indicated. Gelation time is the time at which solution converts to gel form.[16]

Rheological studies

By using Brookfield rheometer or Ostwald's viscometer we can determine the viscosity and rheological properties

of in situ gel. The formulated sample are poured in the small sample adopter of Brookfield viscometer and viscosity was determined using spindle no 18 at different angular velocity. The viscosity was recorded and digitally displayed on the viscometer.[17]

Gelling capacity

In this method 2ml of freshly prepared stimulated tear fluid (STF) are taken in the vials to this add one drop of formulation and equilibrated at 37°C. By visual inspection time taken for the gelation was noted.[18]

Determination of drug content

By using UV visible spectroscopy the drug content and its concentration can be determined. In this method 1ml of the formulated stock solution was taken in 100ml volumetric flask then the volume was made up to 100ml with distilled water. Then 1ml of the above diluted solution was withdrawn and taken in the 10 ml volumetric flask then the volume was made up to 10 ml with distilled water. At 204nm concentration was determined.[19]

Sterility testing

Sterility test was accomplished as per IP. The test was carried out to find out the presence of the fungi, aerobic and anaerobic bacteria by using fluid thioglycolate media and soya casein digest medium under aseptic condition .the formulation were incubated not less than 7 days at 37°C.[20]

Ocular irritancy test

Ocular irritancy test was carried out by HET-CAM test. In this method the egg is placed in the commercial incubators for 10 days at 37°C at relative humidity of about 70%. On the 10^{th} day of evolution the egg is detached from the incubator and candled to control the feasibility of the embryo. A part of each egg shell is removed straight above the air cell and then by using 2-3 ml of 0.9% saline the egg membrane will be carefully moistened and kept in the incubator again. The removed egg were subjected for dosing and continuously observed for lysis for about 5 min, haemorrhaging and coagulation are demonstrated and the extremity of each response after 1 and 5 minutes is documented.[2],[21]

***In vitro* drug release studies**

The *in vitro* drug release studies are accomplish by diffusion process through cellophane membrane using diffusion cell. Then cellophane membrane is soaked in receptor medium that is stimulated tear fluid, pH 6 for overnight. At one end of glass diffusion cell the previously soaked cellophane membrane is tied. The 2ml of the preparation was precisely pipetted into glass cylinder known as donor chamber. The cylinder was then linked to the motor and suspended in the 100ml dissolution medium maintaining the temperature at 37±1°C such that membrane just touches the receptor membrane. The speed was adjusted to 50 rpm. The drug sample was withdrawn at the interval of 30 minutes after withdrawal of sample each sample from receptor medium it was restored by similar volume of the receptor medium. Then the sample was diluted with appropriate receptor medium and examine by UV-visible spectroscopy at 204 nm using receptor medium as a blank.[22]

Accelerate stability studies

The tests for stability were accomplish as per the ICH guidelines. Where this formulated sample were stored in the previously sterilized vial 40±2°C and 75± 5% RH in desiccator placed in a hot air oven to maintain temperature. Sample was withdrawn for every 1 month interval. The % drug content and *in vitro* release studies were carried out. Then the sample was analyzed for pH, clarity and appearance.[23]

RESULT AND DISCUSSION

Ophthalmic in situ gelling system can be formulated by cold method using pluronic f127 as thermosensitve polymer, HPMC K100LV as viscosity enhancing and carbopol 934 as gelling agent in different ratios along with the pure drug Azithromycin which is macrolide antibiotic which is synthetically derived from erythromycin. It is used in the several bacterial infection in the ocular therapy it is mainly used in the treatment of conjunctivitis.

Estimation of Azithromycin by spectrophotometric method

A simple spectrophotometric estimation of Azithromycin was developed by using UV spectroscopy in the stimulated tear fluid, which exhibited λ_{max} at 204 nm in Beer's range of 2-10µg/ml.

FT-IR

FT-IR studies for the pure drug and drug excipient mixture were performed, Pure Azithromycin spectra showed principle peak at different wave number corresponding to its functional group, confirming the purity of the drug as per established standards. The IR Spectra of Azithromycin exhibited peak at 2970.48 cm^{-1}, 1720.56 cm^{-1}, 3255.95 cm^{-1}, 3495.13 cm^{-1}, 1049.31 cm^{-1}, 1485.95 cm^{-1}, (C-H Stretching, C=O Stretching, C-N (3^{o} Amine), OH Stretching, C-O Stretching, C-C Stretching). The IR spectra of drug polymer mixture showed the prominent absorption bands at 3485.49 cm^{-1}, 2901.04 cm^{-1}, 1722.49 cm^{-1}, 1460.61 cm^{-1}(OH Stretching, C-H Stretching, C=O Stretching, C-C Stretching) The FTIR spectra study revealed that the positions of the characteristic absorptions bands for different functional group of the pure drug are slightly changed considerably. Hence concluded that there was no chemical interplay between the pure drug and excipients. (Fig 1)

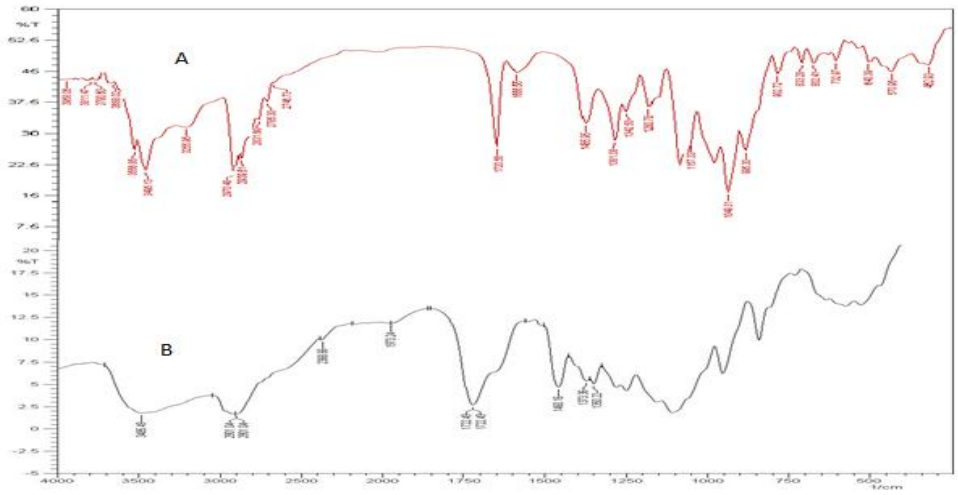

Fig 1: FTIR spectra of Azithromycin (A), and Drug excipient mixture (B).

DSC

DSC studies was accomplish for the pure drug Azithromycin and the excipient mixture (Azithromycin+pluronicf127+HPMC+carbopol) using shimadzu DSC-60 calorimeter at the rate 5.00 °C min^{-1} from 10 °C to 300 °C temperature range under nitrogen flow of 25ml min^{-1}. The DSC of the drug showed a sharp endothermic peak at 131°C which is slightly more than its melting point, indicating the crystallinity. The excipient mixture show the endothermic peak at 135°C. The thermal analysis result obtained show that there was no interplay between the polymer and drug was compatible with all the excipients. (Fig 2)

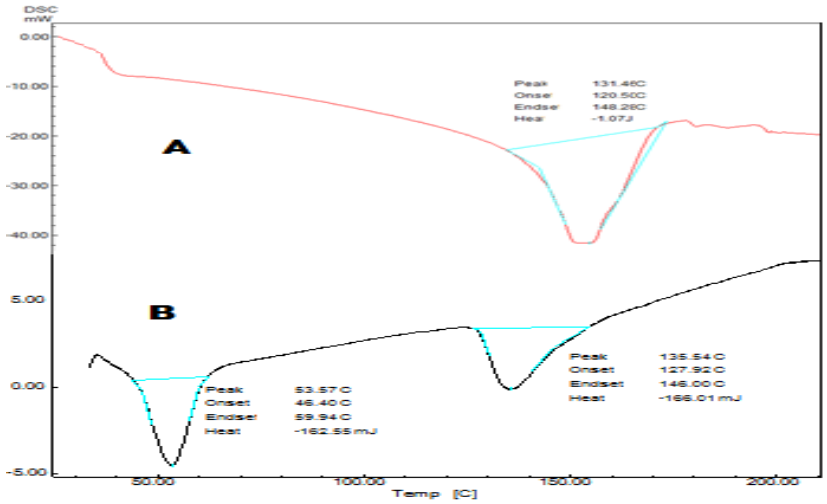

Fig2: DSC thermo gram of Azithromycin (A) and Drug excipient mixture (B).

X-ray diffractometer (XRD)

The XRD pattern of pure Azithromycin and drug polymer mixture was evidenced in Figure 3. X-ray diffraction of Azithromycin showed sharp peaks at 2θ values of 9.7°, 10.6°, 17.4°, 19.2°, 20.4° and 23.9°. The diffraction pattern of Azithromycin and physical mixture showed more intense peaks, but the prominent crystalline peak of the Azithromycin situated at 10.6° was observed. These results confirmed that the drug and excipient does not have interaction between them.

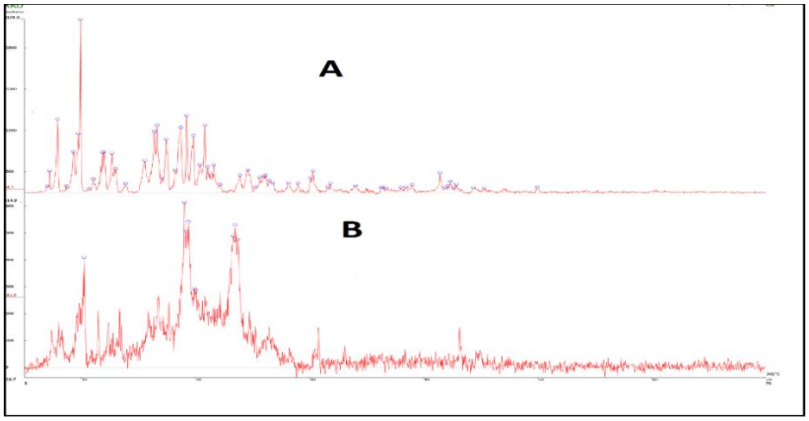

Fig 3: XRD of Azithromycin (A) and Drug excipient mixture (B)

^{1}HNMR

^{1}HNMR studies relate to the functional group involved in the complexation and the chemical shift values in ^{1}HNMR depict the mechanism of complexation. The structural elucidation using ^{1}HNMR showed that the Azithromycin and drug excipient mixture are compatible with each other. These results confirmed that the drug and excipient does not have interaction between them.

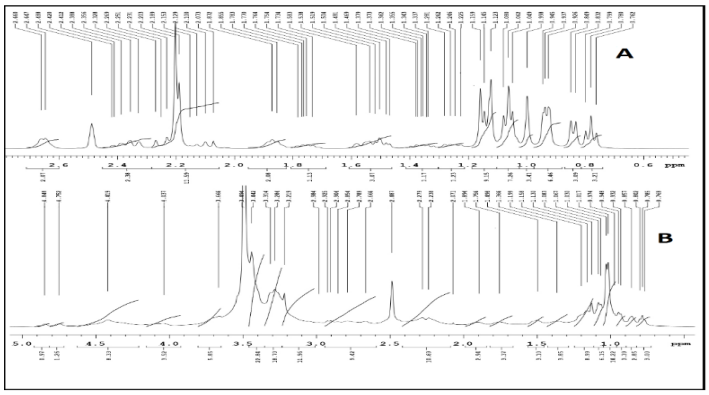

Fig 4: ^{1}HNMR of pure drug Azithromycin (A), ^{1}HNMR of the pure drug with the excipients mixture (B).

Evaluation/depiction of ophthalmic in situ gel

Appearance and clarity

All those prepared formulation of ophthalmic in situ gel were found to be clear and transparent with the help of clarity testing port under black and white background And it was found that there was no sign of any visible particulate matter in the formulations. (Table 2)

pH of gel

The pH of all the formulation lies in between the range of 5.6-6.3 which is under the acceptable range of the ophthalmic in situ gel. It was found that the pH was satisfactory within the range of 6.24-6.55 and it can be suitable for administration. (Table 2)

Sol-Gel transition temperature and Gelation time

Gelation temperature is the temperature at which the solution form of the formulation converts to gel form. Gelation temperature range of the formulations would be 30-36^{0}C. If the gelation temperature lower than 30° C that is in the room temperature it remains in the liquid form when it reaches eye temperature that is at 30-36° C or more than that it get converts to gel form to give sustained drug release. A modulation of the gelation temperature can be achieved by combination of pluronic with other polymer.Increase in the concentration of polymers decreased the gelation temperature.Gelation time was at which gelling occurs.It can be seen that increase in concentration of polymers decreased gelation time. Moreover viscosity of the solution also plays a role for gelation time. Here the optimized formulation had the minimum gelation time and temperature of 1 min and 35°C as compared to other formulations. (Table 2)

Rheological studies

The viscosity of the formulations was determined digitally in viscometer at 37°C, a substantial increase of viscosity was observed, the viscosity values ranges from 18958±6.33 to 25468±4.95 cps were recorded. The All the formulation gives the satisfactory results and it shows that increased in viscosity by increasing temperature and polymer concentration. (Table2)

Gelling capacity

Prepared in situ gelling system were evaluated for *in vitro* gelation capacity. All the formulation give the satisfactory result with the good gelling capacity the +++ symbol shows the indication of good gelation capacity. Which is denoted in the Table 2.

Determination of drug content

The percentage of drug content of Azithromycin from F1-F5 formulation was determined. The results are given in the following table 2. It was observed that the drug content values ranged from 91.70 ±0.985% to 97.92 ±0.809% this implied that drug distribution was uniform and satisfactory in all formulation.

Table 2: Preliminary evaluations of in situ gel.

Evaluation steps	F1	F2	F3	F4	F5
Visual Appearance	Transparent	Transparent	Transparent	Transparent	Transparent
Clarity	Clear	Clear	Clear	Clear	Clear
pH ± SD*	6.24±0.04	6.86±0.03	6.39±0.03	6.65±0.04	6.55±0.03
Gelation temperature (°C)	40	39	37.5	36	35
Gelation time (min) ± SD*	4±0.809	2±0.985	3±0.891	2±0.655	1±0.545
Viscosity(cps) 37^0C± SD*	18958±6.33	22426±5.12	20154±6.18	23426±5.41	25468±4.95
Gelling capacity	+++	+++	+++	+++	+++
Drug Content (%) ± SD*	92.28±0.891	91.70±0.985	94.00±0.854	96.88±0.968	97.92±0.809

***Standard deviation, n=3**

+++ Sign indicates the good gelling capacity which remains for extended period of time.

Sterility testing

All the prepared formulation evaluated for the sterility testing. After 14 days of incubation the results showed that no microbial growth found in all formulation and it was found to be sterile. (Table 3)

Table 3: Sterility testing data of F1-F5 formulation.

Formulations	Days of Incubation						
	Day 1	Day 2	Day 4	Day 6	Day 8	Day 10	Day 14
F1	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-
F4	-	-	-	-	-	-	-
F5	-	-	-	-	-	-	-

-sign indicates the formulation is sterile without any microbial growth.

Ocular irritancy test

Biocompatibility test: - Hen's Egg Test Chorioallantoic Membrane (HET-CAM) test

By doing HET-CAM test we can check for ocular irritancy of the formulation, the illustration of the ocular irritation was observed in the given figure 5 to 8. The test formulation when applied to CAM to controlled substances over a period of 5 minutes, the blood vessels in the CAM go initially from zero after 5 min there was no sign of irritation, lysis, haemorrhages and coagulation on the embryo was observed. It was proved that there was not any ocular irritation effect. Scores showed in the Table 4.

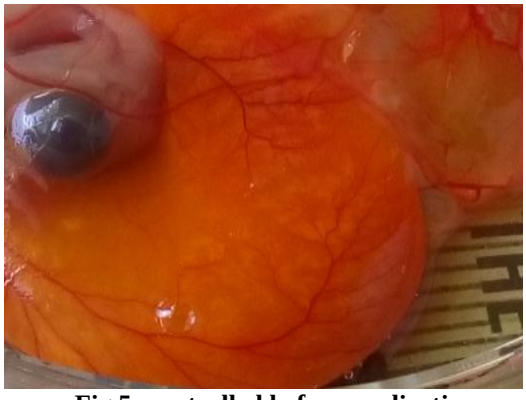

Fig 5: controlled before application.

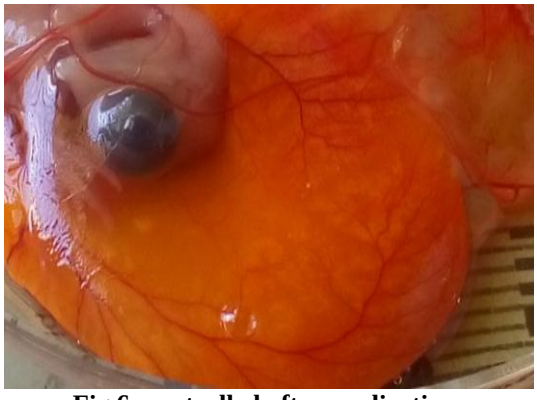

Fig 6: controlled after application.

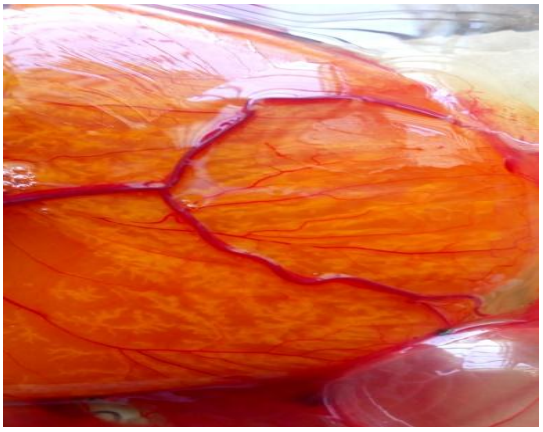

Fig 7: Test before application.

Fig 8: Test after application.

Table 4: Scoring for irritation testing with the HET-CAM test method.

Effect	Time (minutes)		
	0.5	2.0	5.0
Hyperemia	0.0	0.0	0.0
Hemorrhage	0.0	0.0	0.0
Coagulation	0.0	0.0	0.0

***In vitro drug* release studies**

The *in vitro* release of Azithromycin from the formulation was deliberate using cellophane membrane for 24hrs. Result revelled that the all the formulation exhibited biphasic and thus shows the prolonged release of the drug above 86.22%. Thus these increased in the viscosity might have contribution to the decrease in rate of drug release from these formulation. (Fig 9)

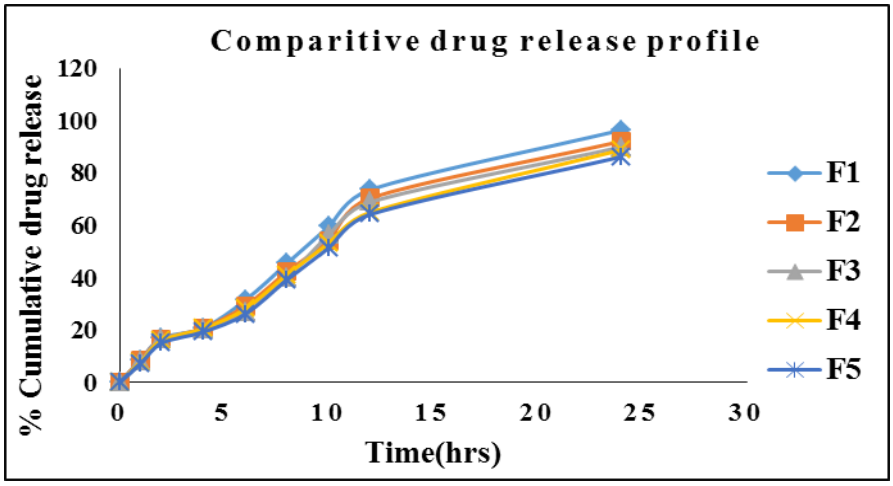

Fig 9: Comparative *In vitro* Release Profile of *in situ* Gel Formulations F1-F5.

Accelerate stability studies

The test for stability was accomplish on the F5at 5°±3°C, 30°±2°C, 65±5% RH and 40°±2°C, 75±5% RH for three months. The formulation were clear no significant changes were observed. This study reveals that there was no definite change observed in the intactness of the drug after subjected to refrigerator (5°±3°C) and room temperature (30°±2°C, 65% ± 5%RH), slight changes shown in the drug content and *in vitro* drug release when subjected to accelerated temperature (40°C, 75±5 % RH) study for 3 months (Table 5, Fig 9 and Fig 10).

Table 5: Accelerated stability studies of F5 formulation of Azithromycin in situ gel.

Sampling condition	Sampling Interval (months)	Physical appearance	% Drug content ± SD*
5±3°C	0	Clear, transparent	97.92±0.759
	1	Clear, transparent	97.89±0.737
	2	Clear, transparent	97.86±0.677
	3	Clear, transparent	97.83±0.648
30±2°C 65% ± 5%RH	0	Clear, transparent	97.92±0.759
	1	Clear, transparent	97.85±0.694
	2	Clear, transparent	97.79±0.625
	3	Clear, transparent	97.68±0.589
40±2°C 75% ± 5%RH	0	Clear, transparent	97.92±0.759
	1	Clear, transparent	95.78±0.503
	2	Clear, transparent	93.53±0.669
	3	Clear, transparent	89.91±0.692

***Standard deviation, n=3**

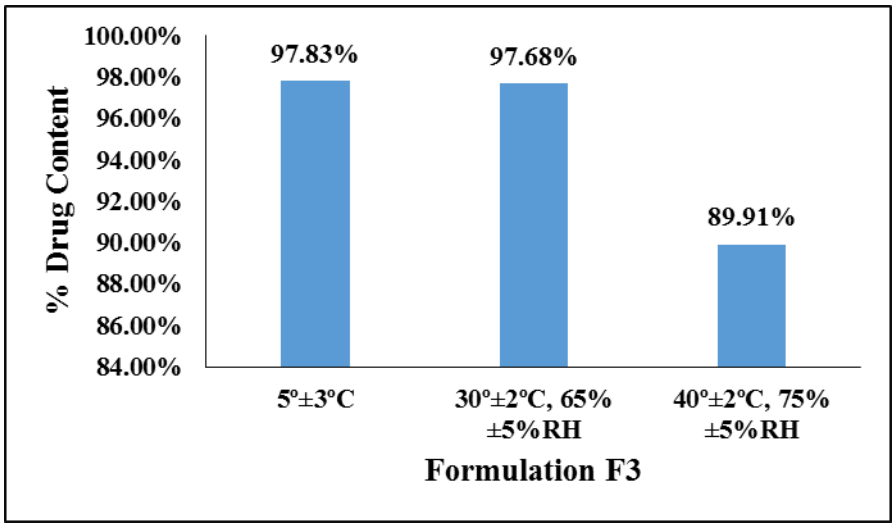

Fig 10: Comparison of % Drug content of F5 Stored at 5°±3°C, 30°±2°C/ 65% ± 5%RH&40°±2°C/ 75% ± 5%RH(after 3 months storage).

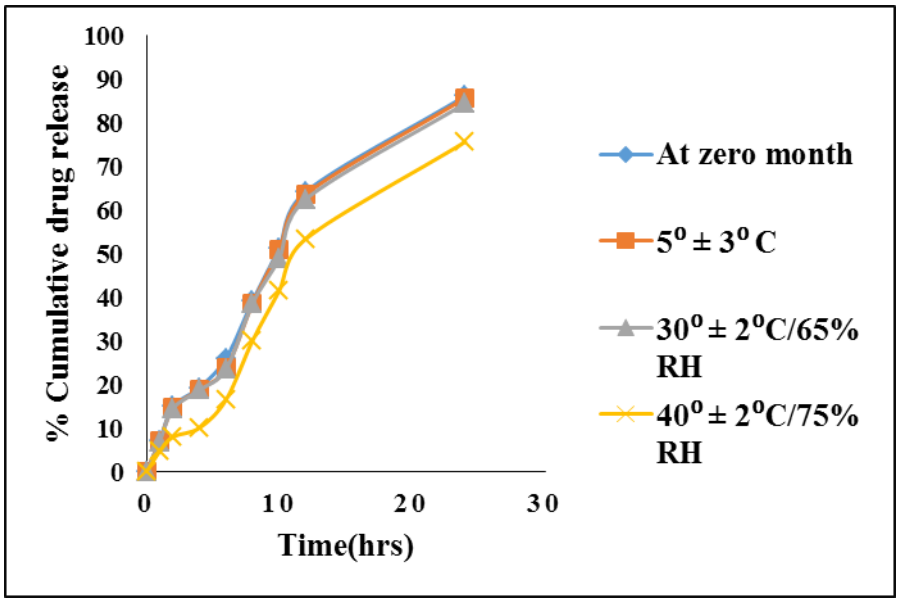

Fig 11: Comparison of *In vitro* drug release profile for formulation F5 at 5°±3°C, 30°±2°C/ 65% ± 5%RH&40°±2°C/ 75% ± 5%RH(after 3 months storage)

CONCLUSION

The purpose of present study is to formulate the ocular drug delivery system in the form of in situ gel to overcome the problem connected with the regular dosage form. In this formulation polymer pluronic F-127, HPMC K-100LV and Carbopol 934 was used with the Azithromycin. Prepared formulation showed the good Sol to Gel property and also showed the required release drug profile along with non-ocular irritant characters. The formulation does not shows any significant changes during the stability testing of 90 days. Hence the developed ophthalmic preparation of Azithromycin represent a viable alternative to the conventional dosage form by improving the pre corneal residential time and enhanced the ocular bioavailability. There by it reduces the frequency of administration for about 24hrs and avoid the drug loss through nasolacrimal drainage resulting in the better patient complaints.

ACKNOLWEDGEMENT

Authors are thankful to the principal, guide of Bharathi College of Pharmacy for providing necessary facilities. Authors are also thankful to the principle, JSS College of Pharmacy, Mysore, India for providing necessary facilities.

REFERENCES

1. Wu Y, Liu Y, Li X, Kebebe D, Zhang B, Ren J, Lu J, Li J, Du S, Liu Z. Research progress of in-situ gelling ophthalmic drug delivery system. Asian J Pharm Sci., 2019; 14(1): 1-5.
2. Laddha UD, Mahajan HS. An insight to ocular in situ gelling systems. Int J Adv Pharm., 2017; 06(02): 31–40.
3. Aligeti SK, Jampala RK, Vinaya J. Formulation and Evaluation of Flurbiprofen Ocular in-Situ Gel. Int J Pharm Sci Res., 2018; 9(5): 1851–6.
4. Vengurlekar p, singh a, rathod s. Microspheric in-situgel for ocular drug delivery system of bromfenac sodium. Int j pharma sci res., 2014; 5(04): 179–85.
5. Bhatia hb, sachan a, bhandari a. Studies on thermoreversive mucoadhesive ophthalmic in situ gel of azthromycin. J drug deliv ther., 2013; 3(05): 106-9
6. Ruel-Gariépy E, Leroux JC. In situ-forming hydrogels - Review of temperature-sensitive systems. Eur J Pharm Biopharm, 2004; 58: 409–26.
7. Kumar S, Haglund BO, Himmelstein KJ. In Situ-Forming Gels for Ophthalmic Drug Delivery. J Ocul Pharmacol, 1994; 10(1): 47–56.
8. Vijaya C, Goud K. Ion-activated in situ gelling ophthalmic delivery systems of azithromycin. Indian J Pharm Sci., 2011; 73(6): 615–20.
9. Akhtar N, Kumar Singh R, Pathak K. Exploring the potential of complex-vesicle based niosomal ocular system loaded with azithromycin: Development of in situ gel and ex vivo characterization. Pharm Biomed Res., 2017; 3(1): 22–33.
10. Avinash k, ajay s. Original research paper formulation and evaluation of thermoreversible in situ ocular gel of clonidine hydrochloride for glaucoma. Pharmacophore [internet]., 2015; 6(5): 220. Available from: http://www.pharmacophorejournal.com/
11. Manimaran V, Damodharan N. Development of fast-dissolving tablets of amlodipine besylate by solid dispersion technology using poloxamer 407 and poloxamer 188. Asian J Pharm Clin Res., 2017; 10(7): 135–41.
12. Ghahremankhani AA, Dorkoosh F, Dinarvand R. PLGA-PEG-PLGA tri-block copolymers as in situ gel-forming peptide delivery system: Effect of formulation properties on peptide release. Pharm Dev Technol, 2008; 13(1): 49–55.
13. Gupta C, Juyal V, Nagaich U. Formulation and optimization of thermosensitive in-situ gel of moxifloxacin hydrochloride for ocular drug delivery. Int J Appl Pharm, 2018; 10(3): 123–30.
14. Vyshnavi V, Indira S, Srinivas P. Formulation and Evaluation of Nasal Niosomal in situ Gels of Loratadine.Int J Pharm Sci Drug Res. CODEN (USA)., 2015; 7(1): 13–21.
15. Alfadhel K, Al-Mulla A, Al-Busairi B. Development and characterization of novel polybutylene nanocomposites. J Compos Mater., 2017; 51(1): 95–108.
16. Madan M, Bajaj A, Lewis S, Udupa N, Baig JA. In situ forming polymeric drug delivery systems. Vol. 71, Indian J Pharm Sci., 2009; 71(3): 242–51.
17. Sampathi S.Development and Characterization in Situ Gels of Lomefloxacin Hydrochloride for Ocular. World J Pharm Pharm Sci., 2014; 3(2): 2350–64.
18. Saxenaa P, Kushwaha SKS. Temperature sensitive ophthalmic hydrogels of Levofloxacin hemihydrate with enhanced solubility and prolonged retention time. Int J Pharm Pharm Sci., 2013; 5(3): 877–83.
19. Gupta SK, Singhvi IJ. Sustained ophthalmic delivery of moxifloxacin hydrochloride from an pH triggered in situ gelling system. Res J Pharm Technol, 2012; 5(12): 1538–42.
20. Geethalakshmi A, Mahalingan K, Jha SK. In vitro characterization & Pharmacokinetic evaluation of ion activated in situ gelling system for Betaxolol Hydrochloride.J Innov Pharm sci., 2017; 1(1): 6–15.
21. Rajpal Deshmukh G, Hema Kumar K, Suresh Reddy PV, Srinivasa Rao B, Venkata Satish Kumar C. Evaluation of Eye Irritation Potential of Aqueous Leaf Extract of Achyranthes aspera by In Vitro and In Vivo Method . ISRN Toxicol, 2012; 2012: 1–5.
22. Mandal S, Thimmasetty MKMJ, Geetha MS. Formulation and evaluation of an in situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride.Int J Pharm Investig, 2012; 2(2): 78-82.
23. Garala K, Joshi P, Patel J, Ramkishan A, Shah M. Formulation and evaluation of periodontal in situ gel. Int J Pharm Investig, 2013; 3(1): 29-41.