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Sustain Ophthalmic Delivery of Azithromycin from pH-Induced *in Situ* Gelling system

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ABSTRACT

Most eye diseases are treated with the topical application of eye drops. The poor bioavailability and therapeutic response exhibited by these conventional eye drops due to rapid precorneal elimination of the drug may be overcome by the use of in situ gelling system that are instilled as drops in the eye and undergo a sol-to-gel transition in the cul-de-sac. Hence the purpose of the present work was to develop Sustain Ophthalmic Delivery of Azithromycin form pH induced in situ gelling system. Polyacrylic acid (carbopol 934) was used as gelling agent in combination with hydroxy propyl methyl cellulose (HPMC K 100 LV) which acts as a viscosity enhancing agent. Compatibility studies of the drug excipients were carried out using FTIR and DSC. The prepared formulation were characterized for clarity, pH, drug content, gelling capacity, in vitro drug release study, sterility study and stability study. Experimental part showed that viscosity of sols was increased with increase in the concentration of polymers and the solutions shown pseudo plastic behavior. The antimicrobial studies against Staphylococcus aureus and in vivo ocular irritancy studies using suitable animal models were performed. All the results were found to be satisfactory. The formulations were therapeutically efficacious, sterile and provided sustained release of the drug over a period of time. These results demonstrate that the developed system is an alternative to conventional drug delivery system, patient compliance, industrially oriented and economical.

Key words: *Azithromycin, In situ gelling, ophthalmic delivery system, Carbopol 934, HPMC.*

INTRODUCTION

The eye is a sensory organ that converts light to an electric signal that is treated and interpreted by the brain. Briefly, the eye possesses efficient protective mechanisms like reflex blinking, lachrymation, and drainage, while lid closure protects the eye from external aggression. Tears permanently wash the surface of the eye and exert an anti-infectious activity through the lysozyme and immunoglobulin's they contain. Finally, the lachrymal fluid is drained down the nasolacrimal pathways. All these protective mechanisms are responsible for therapist and extensive precorneal loss of topically applied ophthalmic drugs [1]. *In situ* is a Latin word which means 'In its original place or in position'. There are many mechanisms which triggers the formulation of *in situ* gels such as solvent exchange, ultra violet irradiation, ionic cross linkage, temperature modification, pH change and ionization. Studies are performed through various routes like oral, rectal, ocular, injectable, vaginal, nasal, parental and intraperitoneal. With the increased demand in techniques and recent developments in the field of polymer sciences various stimuli sensitive hydrogels like pH and temperature sensitive hydrogels are developed, which are used as chemotherapeutic agents to tumour regions. Prolonged and sustained release of the drug, reproducible, excellent stability, biocompatible and accurate quantities of administration makes the *in situ* gel system more reliable. *In situ* gel formulation applied for targeted delivery via ophthalmic, rectal, vaginal, nasal mucosa avoids the hepatic first-pass metabolism, especially for the proteins and peptides [2]. The development of *in situ* gel systems has received considerable attention over the past few years. *In situ* gel forming drug delivery systems are principle, capable of releasing drug in a sustained manner maintaining relatively constant plasma profiles. These hydrogels are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH. These have a characteristic property of temperature dependent, pH dependent and cat ion induced gelation [3]. The aim of present work is to prepare *in situ* gel to increase the precorneal residence time. A combination of carbopol and HPMC was investigated as a vehicle for the formulation of pH triggered *in situ* gel when instilled to the eye provide sustain release of drug. With simulated tear fluid (In proportion of 25:7 ie, application of volume 25µl and normal volume of tear fluid in the eye 7µl) to find out the gelling capacity of the ophthalmic product. Azithromycin is sparingly soluble in water and neutral pH-7 in order to increase the solubility of Azithromycin. Azithromycin is a macrolide antibiotic agent synthetically derived from erythromycin. It is used in the treatment of various infections caused by bacteria. Azithromycin has been proven effective for the treatment of trachoma.

Overview of Anatomy and Physiology of Human Eye:

The eye is vital organ of human body. The cornea, lens, and vitreous body are transparent media with no blood vessels. Oxygen and nutrients are transported to these nonvascular tissues by the aqueous humours. The aqueous humours has a high oxygen tension and about the same osmotic pressure as blood. The cornea also derives part of its oxygen need from the atmosphere and is richly supplied with free nerve endings. The transparent cornea is continued posterior into opaque white sclera, which consists of tough fibrous tissue. Both cornea and sclera withstand the intraocular tension constantly maintained in the eye. The eye is constantly cleansed and lubricated by the lachrymal apparatus, which consists of four structures: Lachrymal glands, lachrymal canals, lachrymal sac, naso-lachrymal duct. The lachrymal fluid secreted by the lachrymal glands is emptied on the surface of the conjunctiva of the upper eyelid at a turnover rate of 16% per min. It washes over the eyeball and is swept up by the blinking action of the eyelids. Thus the eyeball is continually irrigated by a gentle stream of lachrymal fluid that prevents it from becoming dry and inflamed. The lachrymal fluid in humans has a normal volume of 7µl and is an isotonic aqueous solution of bicarbonate and sodium chloride (pH 7.4) that serves to dilute irritants or to wash the foreign bodies out of the conjunctival sac. It contains lysozyme, whose bactericidal activity reduces the bacterial count in the conjunctival sac. The rate of blinking varies widely from one person to another, with an average of approximately 20 blinking movements per min. During each blink movement the eyelids are closed for a short period of about 0.3 sec [4].

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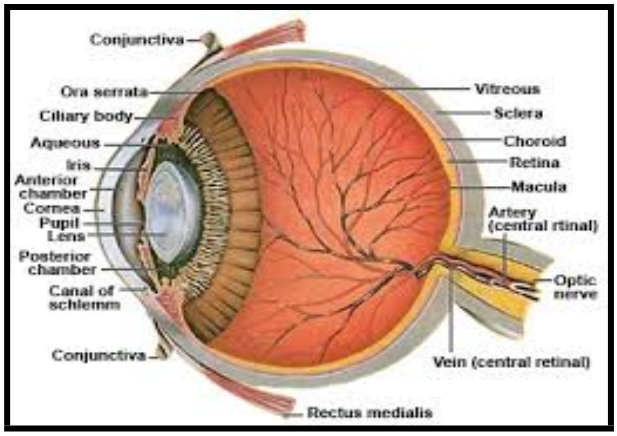

Fig. 1: Overview of Anatomy and Physiology of Human Eye

Fate of the Formulation Administered Through Ocular Route:

At first sight, the eye seems an ideal, easily accessible target organ for topical treatment. However, the eye is, in fact, well protected against absorption of foreign materials, first by eyelids and tear-flow and then by the cornea, which forms the physical-biological barrier. When any foreign material or medication is introduced on the surface of the eye, the tear-flow immediately increases and washes it away in a relatively short time. Under normal conditions, the eye can accommodate only a very small volume without overflowing. This anatomy, physiology and biochemistry of the eye are responsible for the low bioavailability of drug. The challenge is to overcome these protective barriers of the eye without causing permanent tissue damage [5].

Advantages: [6]

1. Flexibility in dosing / accurate dosing.
2. Sustained and controlled drug delivery possible.
3. Increased ocular bioavailability of drug (by increased corneal contact time).
4. Ability to circumvent the protective barriers like drainage, lacrimation and conjunctival absorption.
5. Needle-free drug application, self-medication possible, better patient compliance.
6. Avoidance of hepatic first pass metabolism and thus potential for dose reduction compared to oral delivery.

Limitations: [6]

1. Interference with vision.
2. Difficulty in placement and removal.
3. Limited permeability of cornea resulting into low absorption of drugs.
4. Entry of drug to the lachrymal duct may cause unwanted systemic side effects.
5. The elimination of the drug through the eye blinking and tear flow may lead to short duration of the therapeutic effect resulting in a frequent dosing regimen.

MATERIALS AND METHODS

Materials:

Azithromycin, Hydroxypropylmethyl cellulose (HPMC) and HPMC K15M were obtained from SD Fine Chemicals Limited, Mumbai, India and Carbopol 934 was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. All other chemicals/reagents used were of analytical grade, available commercially and used as such without further processing. A UV/Vis spectrophotometer (Systronics, Double beam UV-VIS Spectrophotometer: 2201) was used for drug analysis.

Compatibility study:

Compatibility studies were carried out in order to establish that, there would be no interaction between the drug and excipients (Polymers) used in the formulation. These studies were carried out by Perkin Elmer FTIR, Spectrum BX, by a powder method and DSC.

1. Fourier transforms infra-red spectroscopy (FT-IR) analysis:

The FT-IR spectra of the drug with polymers were compared with the standard FT-IR spectrum of the pure drug. In this study, the compatibility of the pure drug Azithromycin with the polymers Carbopol 934, HPMC K 100LV prior to the preparation of ophthalmic *in situ* hydrogel is carried out [7]. (Fig. 2 & 2.1)

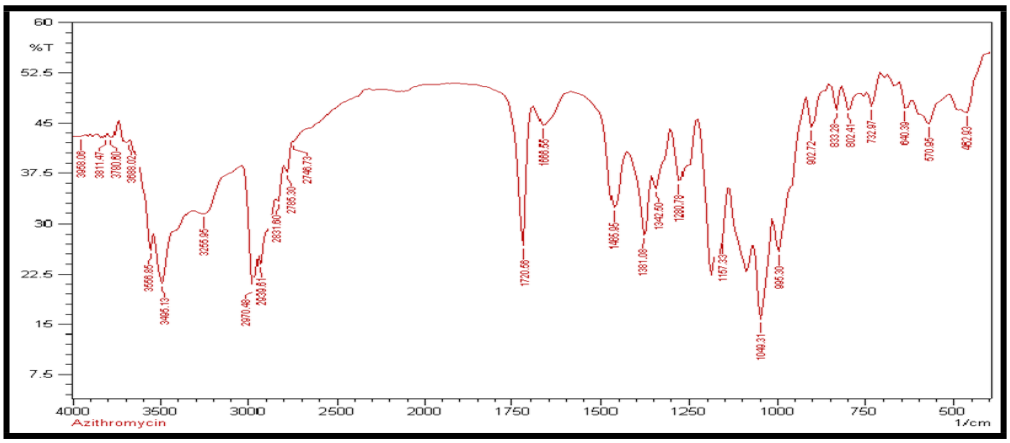

Fig. 2: FT-IR spectra of pure Azithromycin

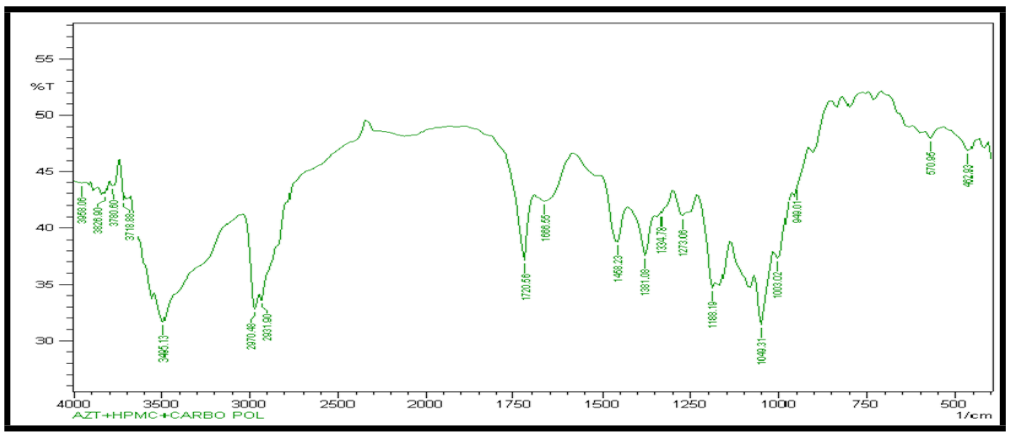

Fig. 2.1: FT-IR spectra of Azithromycin+HPMC+Carbopol

2. Differential scanning colorimetry (DSC):

The DSC analysis of pure drug and drug, HPMC, Carbopol were carried out using a Diamond DSC (PerkinElmer, USA) to evaluate any possible drug polymer interaction.9 The analysis was performed at a rate 5.00 ºC min $^{-1}$ from 10 ºC to 300 ºC temperature range under nitrogen flow of 25ml min^{-1}(Fig. 2.2 & 2.3)

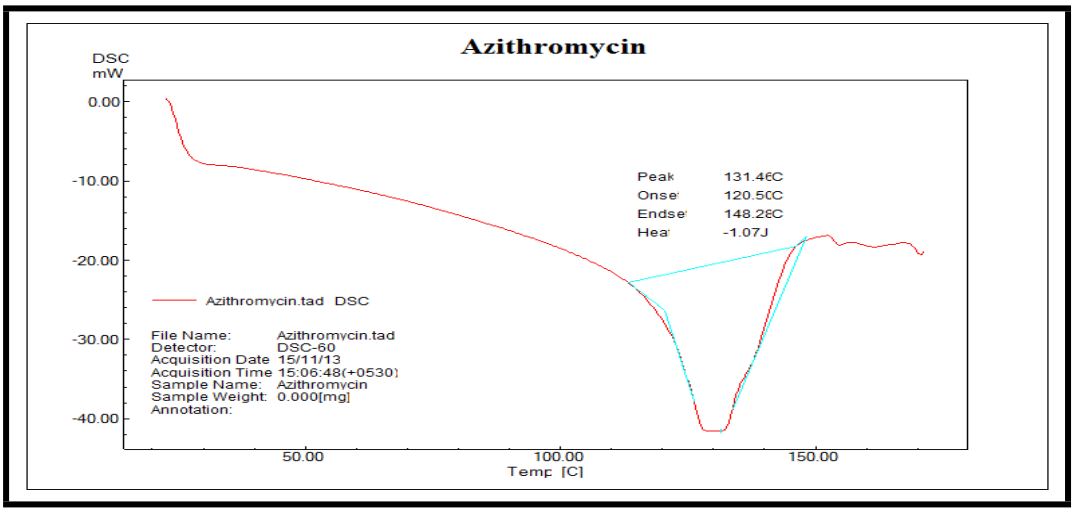

Fig. 2.2: DSC of Azithromycin

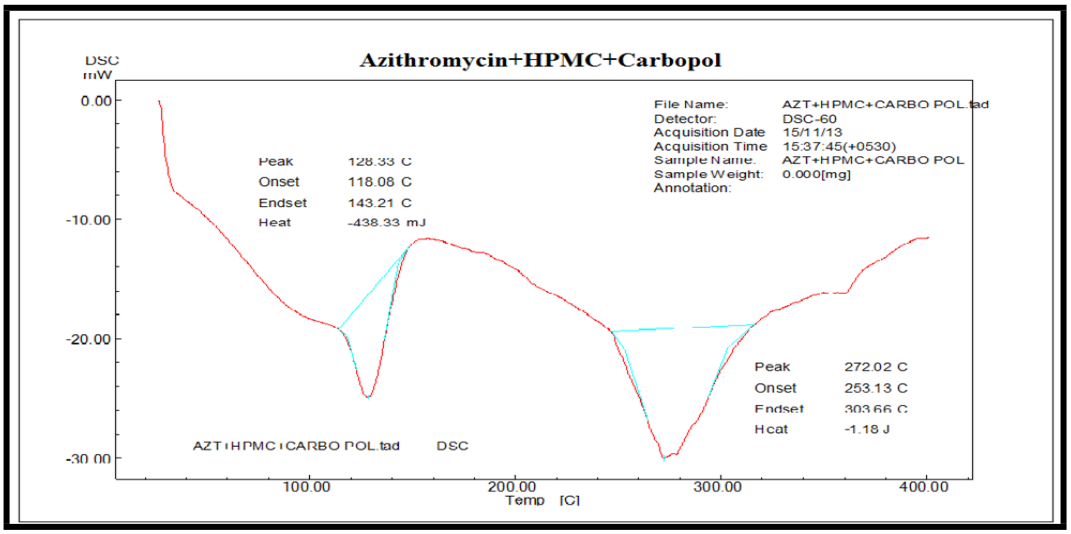

Fig. 2.3: DSC of Azithromycin+HPMC+Carbopol

Method of preparation:

Take 70ml of Online water for injection in 100ml of glass beaker. Heat for 10 min until it boils. Then add HPMC in small partwise with full stirring with an overhead stirrer. After complete addition of HPMC, the solution is allowed to cool in cold water (ice). It is clear, colourless, viscous solution. Now, add and dissolve citric acid and di-sodium hydrogen phosphate. Then Azithromycin was dissolve in 100ml of Acetate buffer and dissolve it separately. The drug solution was then added to the polymeric solution under constant stirring until a uniform solution was obtained. Now, add carbopol 934 small partwise and stir for dissolution and then add Tween 80 and heat it. Then after complete dissolution add Benzalkonium chloride as per calculation. Now, WFI was added to make up the volume to 100ml of the solution, is filter through filter paper by applying Vacuum. The formulations were filled in the vials under aseptic conditions and evaluations were carried out. Method of preparation which are mentioned in table 1.

Table No. 1: Optimized formula table of Azithromycin for pH-induced *in situ* gelling system

SI No.	Ingredients	Formulations				
		F1	F2	F3	F4	F5
1	Azithromycin (%w/v)	0.5	0.5	0.5	0.5	0.5
2	Carbopol 934 (%w/v)	0.1	0.2	0.3	0.4	0.5
3	HPMC 100LV (%w/v)	1.5	1.5	1.5	1.5	1.5
4	Citric acid (%w/v)	0.407	0.407	0.407	0.407	0.407
5	Di-sodium hydrogen phosphate (%w/v)	1.125	1.125	1.125	1.125	1.125
6	Tween 80 (ml)	0.5	0.5	0.5	0.5	0.5
7	Benzalkonium chloride (%w/v)	0.005	0.005	0.005	0.005	0.005
8	WFI (ml) qs to	100	100	100	100	100

Composition of simulated tear fluid (STF):

The composition of simulated tear fluid used was sodium chloride 0.670g, sodium bi carbonate 0.200g, calcium chloride 0.008g, Purified water q.s 100 ml [8].

Evaluations of Formulation:

1. Test for clarity & Appearance:

The clarity of the formulation was determined by black and white background. The vials were held horizontally and gently rotated immediately under the lamp and inverted once or twice to

detect foreign particle. The appearance was determined by visually [9].

2. Determination of pH:

The pH of all formulation was determined immediately after preparation as well as after 24 hours of storage at Refrigerator with the help of digital pH meter [10]. (Table 1.1)

3. Gelling capacity:

The gelling capacity was determined by placing a drop of the formulation in a test-tube containing 2ml of simulated tear fluid (STF) freshly prepared and equilibrated at 37°C. The visual assessment of gel formulation was carried out simultaneously. The time required for gelation as well as time taken for the formed gel to dissolve was also noted.

The flow behaviour with the "+" sign indicates the vehicle is in the liquid form which show gel slowly and dissolve rapidly.

The flow behaviour with the "++" sign indicates the vehicle is in the liquid-gel like form and flows less readily, which shows gelation immediately and remains for a few hours.

The flow behaviour with the "+++" sign indicates the vehicle is in the gel form and is very difficult to flow which also show immediate gelation and the gel remains for the extended period of time [8]. (Table 1.1)

4. % Drug content:

Uniform distribution of active ingredient is important to achieve dose uniformity. The drug content was determined by diluting 1ml of the formulation to 100ml with simulated tear fluid pH 7.4. Aliquot of 5ml was withdrawn and further diluted to 25ml with STF. Azithromycin concentration was then determined at 235nm by using UV-Vis spectrophotometer [10, 11]. (Table 1.1)

Table No. 1.1: Result for Evaluation Parameters

Formulation code	Appearance	pH	Gelling Capacity	%Drug content
F1	Clear	6.19	+++	92.27%
F2	Clear	6.25	+++	91.79%
F3	Clear	6.39	+++	96.84%
F4	Clear	6.20	+++	94.00%
F5	Clear	6.24	+++	97.94%

5. Anti-Microbial Efficiency studies:

The microbiological studies were carried out to ascertain the biological activity of the optimized formulation and compared with marketed eye drops against microorganism (Staphylococcus aureus) applying "cup plate method". A layer of nutrient agar (20ml) was seeded with 0.2ml of test micro-organism and 7µl of STF and allowed to solidify in the petriplate. Cups were made with the help of sterile borer at 4 mm diameter on the solidified agar layer. The formulations, 25µl of each were poured into respective cups in an aseptic condition. These plates were kept in incubator for a period of 24 hrs and then observed for zone of inhibition [12]. (Fig. 3)

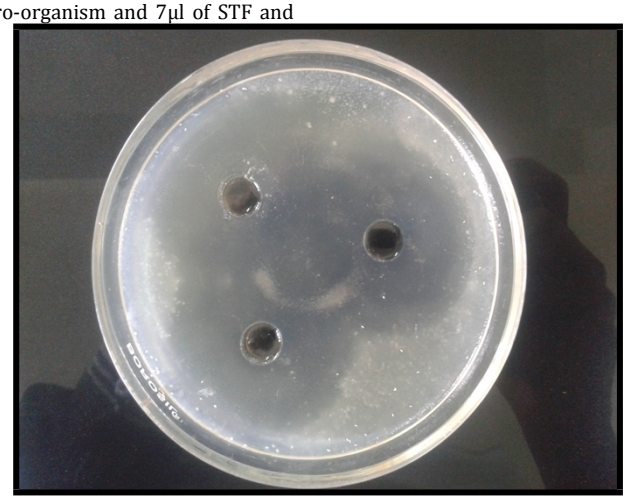

Fig. 3: Anti-microbial activity

6. Measurement of Surface tension:

Surface tension was measured by Stalagmometer by drop count method. The numbers of drops were counted and calculated surface tension [13].

7. Determination of viscosity of the ophthalmic formulation:

Viscosity of the formulated solution and gel were measured by Brookfield DVE digital viscometer. Guard leg was mounted on the viscometer. Helipath spindle no.18 was used for measurement of viscosity of solution. Helipath spindle was inserted in the test material until fluid level was at the immersion groove on the spindle. The spindle was attached to the lower shaft of the viscometer. The shaft was lifted slightly; holding it firmly with one hand while screwing the spindle. The spindle code 00 was used for measurement of the viscosity of solution and spindle code S 18 was used for measurement of the viscosity of gel. The motor was turn on and spindle was rotated. The viscosity was noted from the display window and the readings were recorded [13]. (Table 1.2)

Table No. 1.2: Physicochemical Evaluation Parameters of the formulations

Formulation Code	Density (g/ml)	Surface tension (dynes/cm)	Viscosity (Poise)
F1	0.9780	57.684	0.09180
F2	0.9774	56.654	0.09602
F3	0.9770	56.630	0.08722
F4	0.9782	54.810	0.08522
F5	0.9800	54.011	0.09346

8. Sterility test:

All ophthalmic preparations should be sterile therefore the test for sterility is very important evaluation parameter. The sterility test was performed according to the Indian pharmacopoeia.

Direct inoculation technique was used for sterility testing of the ophthalmic solutions. Sterile Fluid Thioglycolate and Soya bean Casein media were used to detect bacteria and fungi growth respectively. Medias were sterilized by moist heat sterilization

technique. One set of positive control (Bacillus Subtilis and Aspergillus Niger) and negative control for each medium were used for the comparative study. 2ml of the liquid form test container was removed with a sterile pipette or with a sterile syringe. The test liquid was aseptically transferred to fluid thioglycolate medium (20ml) and soyabean-casein digest media. The inoculate media was incubated for not less than 14 days at 30ºC in the case of thioglycolate medium and 20ºC in the case of soyabean-casein digest medium [14]. (Table 1.3)

Table No. 1.3: Sterility testing data for *in situ* ophthalmic gel

Formulation Code	Days of incubation	Thioglycolate medium	Soyabean-casein digest medium
F1	14	-	-
F2	14	-	-
F3	14	-	-
F4	14	-	-
F5	14	-	-

9. Ocular irritancy studies:

The Draize technique was designed for the ocular irritation potential of the ophthalmic product[15]. According to the Draize test, the amount of substance applied to the eye is normally 100µl placed into the lower cul-de-sac with observation of the various criteria made at a designed required time interval of 1hr, 24hrs, 48hrs, 72hrs, and 1week after administration [15, 16]. Three male rabbits weighing 1.5 to 2kg were used for the present study. The sterile formulation was instilled twice a day for a period of 7 days, and a cross-over study was carried out (with a 3 day washing period with saline was carried out before the cross-over study). Rabbits were observed periodically for redness, swelling, watering of the eye. (Fig. 3.1)

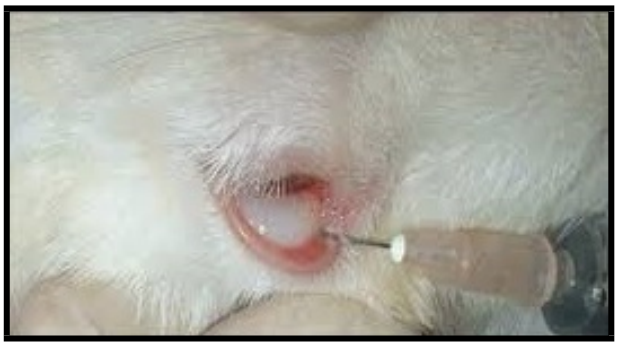

Fig. 3.1: Ocular irritancy study

10. In vitro Drug release Studies:

In vitro release studies were carried out using bi-chambered donor receiver compartment model (Franz diffusion cell) *in vitro* release of Azithromycin was carried out in formulation with using cellophane membrane. The cellophane membrane previously soaked overnight in the dissolution medium. The dissolution medium 100ml of simulated tear fluid stirred 50rpm at 37ºC ±0.5ºC one end of the dissolution tube was covered by a cellophane membrane. The 1ml of formulation were spread on the cellophane membrane and membrane was placed such that it just touches the diffusion medium [STF] present in receptor compartment. Aliquots each 1ml volume were withdrawn at hourly intervals and replaced by an equal volume of receptor medium. The drug sample were withdrawn at the interval of one hour for the period of 8hrs from diffusion medium and analysed by a UV spectrometer at 235nm using simulated tear fluid as blank [17-19]. (Fig. 3.2)

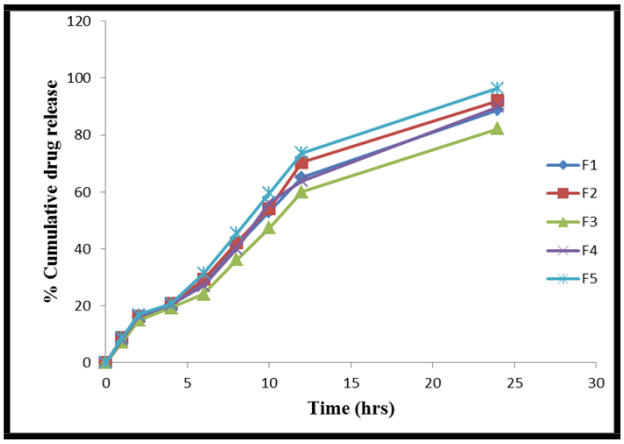

Fig. 3.2: *in vitro drug release of in situ* ophthalmic gel of Azithromycin

11. Release kinetics studies:

To analyse the mechanism for the release kinetics of the dosage form, the data obtained was fitted to Zero order, First order, Higuchi matrix and Peppas model. After comparing the r-value, the best fit model was selected [18, 20]. (Table 2)

Table No. 2: Correlation coefficients according to different kinetic equations.

Formulations	%Cumulative drug release	Zero order	First order	Higuchi plot	Peppas plot	'n' values
F1	88.88	0.9427	0.9836	0.8958	0.8219	1.1869
F2	92.14	0.9350	0.9745	0.8909	0.8083	1.1811
F3	82.22	0.9466	0.9821	0.8821	0.8228	1.1608
F4	89.97	0.9420	0.9800	0.8878	0.8123	1.1771
F5	96.47	0.9289	0.9641	0.8967	0.8138	1.2055

F1, F2, F3, F4 and F5 represent formulations 1 to 5 respectively, etc.

12. Stability studies:

Stability testing of the ideal formulation was kept at 5ºC, 30ºC and 40ºC for the period of 90 days. Three containers of each formulation type were used. The samples were analysed for their appearance, pH, viscosity, gelation nature, drug content in vitro drug release at the end of test period and the results were recorded [17, 18]. (Table 2.1)

Table No. 2.1: Stability Studies: *In vitro* release study of formulation F3 after 3 months of storage at 5ºC, 30 & 40ºC

Time (hrs)	%Cumulative Drug release		
	5ºC	30ºC	40ºC
0	0	0	0
1	7.03	6.93	4.73
2	14.64	14.56	8.06
4	18.92	18.78	10.11
6	23.78	23.62	16.62
8	35.69	35.67	27.17

10	47.02	46.88	38.58
12	59.62	59.55	47.45
24	81.74	81.51	72.71

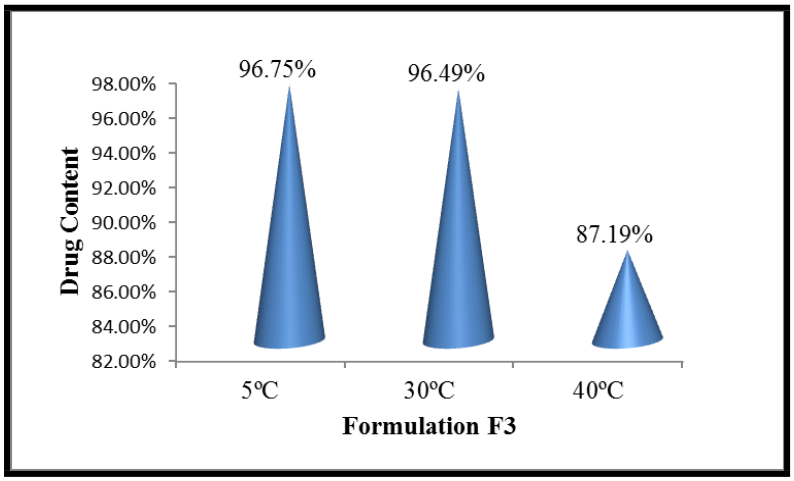

Fig. 3.3: Stability study: Comparison of %Drug content of formulation F3 at 5ºC, 30 and 40ºC ± 2 ºC.

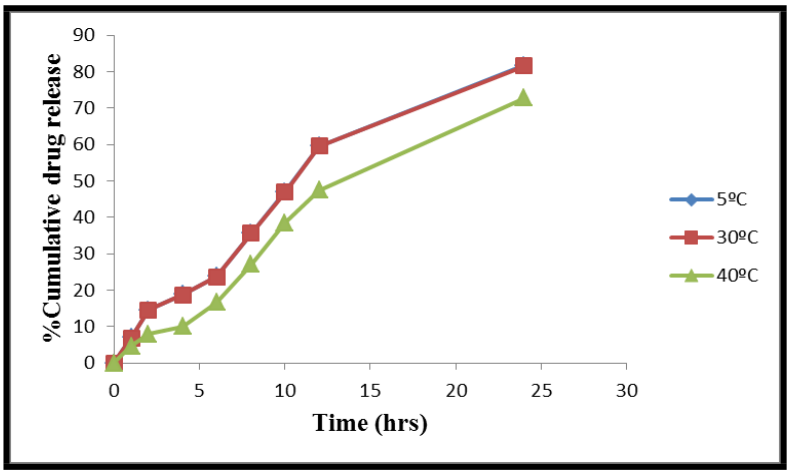

Fig. 3.4: Stability study: Comparison of *In vitro* drug release profile for formulation F3 at 5ºC, 30 & 40ºC (after 3 months of storage as per QAI(R))

RESULT AND DISCUSSION

Ophthalmic *in situ* gelling system can be formulated using Carbopol 934 as pH-triggered polymer along with HPMC K 100LV as viscosity enhancing agent. The identification and characterization of Azithromycin shows it complies with standard. Infra-red spectroscopy and differential scanning colorimetry of Azithromycin, Carbopol and HPMC K 100LV alone and there physical mixture revealed that, Azithromycin is compatible with the entire polymer used. The clarity of the formulations was found to be satisfactory. Terminal sterilization by autoclaving had left no effect on the clarity and other physicochemical properties of the formulations, no changes were observed after autoclaving. pH of the formulation was found to be satisfactory and lies in the range of 6-7.4. Gelling capacity, %Drug content acceptable for all five formulations, but the formulation F3 shows better result. In this study, formulation F3 shows better rheological characters. *In vitro* drug release study, of all five formulations shows sustained drug release for a period of 24hrs and follows non-fickian drug release. In sterility study there was no appearance of turbidity and hence no evidence of microbial growth in the formulation. From this study, formulation F3 was selected as optimized formulation.

CONCLUSION

Modern research in novel approach in drug therapy is focused I maximizing the therapeutic efficacy of the drug. Many polymers have been used to fulfil the objectives of sustain release of the drug through ocular drug delivery to prevent the loss of drugs through tears. The present work was aimed at fabricating the evaluating ophthalmic drug delivery of *in situ* gel of Azithromycin.

Azithromycin is a macrolide antibiotic agent synthetically derived from erythromycin. It is used in the treatment of various infections caused by bacterial agent used in the treatment of ocular infection was successfully formulated as *in situ* gel using polymer. With increasing the concentration of polymers carbopol and HPMC, which was used as a phase transition element for sustaining the diffusion of Azithromycin, it was observed that the onset of gelation was faster the gelling system were prepared using (Acetate buffer 5.0) in order to examine the effect of pH on release profile of the drug. The samples showed no significant changes during the period of 90 days and are stable. The formulated system provided sustained release of the drug for more than 24hrs. The developed formulation is viable alternative to conventional eye drops due to its ability to enhance bioavailability through the longer precorneal residence time and ability to sustain the drug release. Also important of ease of administration and decrease the frequency of administration resulting in better patients compliance.

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