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DESIGN, DEVELOPMENT AND EVALUATION OF CONTROLLED RELEASE OCULAR MINITABLETS OF FLUOROQUINOLONES

Phadke Ninad Mukund[1*], Phadtare Dipti Ganesh[2], Saudagar Ravindranath Bhanudas[3]

[1]Department of Quality Assurance Techniques, KCT'S RGS College of Pharmacy, Anjaneri, Nasik, 422 213. Maharashtra, India

[2]Department of Quality Assurance Techniques, KCT'S RGS College of Pharmacy, Anjaneri, Nasik, 422 213. Maharashtra, India

[3]Department of Pharmaceutical Chemistry, KCT'S RGS College of Pharmacy, Anjaneri, Nasik, 422 213. Maharashtra, India

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***Correspondence for Author**
Ninad Phadke
Department of Quality Assurance Techniques, KCT'S RGS College of Pharmacy, Anjaneri, Nasik, 422 213. Maharashtra, India

ABSTRACT

Transport of drugs applied by traditional dosage forms is restricted to the eye, and therapeutic drug concentrations in the target tissues are not maintained for a long duration since the eyes are protected by a unique anatomy and physiology. There is urgent need to develop ocular drug delivery systems which provide controlled release for the treatment of chronic diseases, and increase patient's and doctor's convenience to reduce the dosing frequency and invasive treatment. This research was carried out to develop Ocular Minitablets which are novel ocular dosage forms for the treatment of the posterior segment of the eye that produced controlled release of drug for the period of 12 hrs. Basically these formulations are nothing but ocular inserts prepared by direct compression technique. Levofloxacin, Carbopol 971G, HPMC K4M, HPMC K100 M, Sodium CMC and magnesium stearate were used for preparation of ocular minitablets. Antibacterial studies showed that the Levofloxacin ocular minitablets are best suited for the treatment of bacterial keratitis or conjunctivitis.The formulations were found to be non-irritating with no ocular damage.

KEY WORDS: Ocular Minitablets, Levofloxacin Hemihydrate, Bacterial Keratitis, Bacterial Conjunctivitis.

1. INTRODUCTION

The eye is a unique organ that distinguishes itself from other parts of the body rather distinctively. It is protected physically by tough layers of sclera and cornea and is isolated anatomically by blood eye barriers. Eye disorders, especially posterior segment diseases, are difficult to treat as the eye is usually regarded by patients as being sensitive and there may be unwillingness on that part to use some of the more in invasive treatments available[(1)]. There are at least three major factors that have to be considered when drug delivery to the eye is attempted. First, is how to cross the blood eye barrier or cornea to reach the intraocular site. Secondly, how to localize drug actions at the eye without affecting other parts of the body. Finally how to prolong the duration of drug actions such that the frequency of drug administration can be minimized[(2)]. In order to maintain an effective concentration in ocular tissues, rather high concentrations or doses of a drug have to be administered. If these high doses are administered systemically, untoward and undesirable side effects will be manifested.However, poor ocular bioavailability of drugs has remained one of the greatest challenges for ophthalmologists treating these clinical conditions. Consequently, a local topical instillation is preferred over systemic administration for drug delivery to the eye. There are different ocular delivery systems have been developed to overcome the bioavailability problems faced by the traditional ophthalmic preparations as eye drops or ointments, also to prolong and continue the drug release from the ophthalmic dosage form, and hence decrease the more frequent dosing regimen of most of the conventional used systems[(3)]. A basic concept in ophthalmic research and development is that the therapeutic efficacy of an ophthalmic drug can be greatly improved by prolonging its contact with the corneal surface. Various ocular novel drug delivery systems such as in-situ gelling systems, Ocular inserts, liposomes, niosomes, Ocular Minitablets.[(4)] Ocular minitablets are a relatively new innovation in the treatment of ocular disease. These are extremely small tablets, approximately 2-8 mm in diameter and with an average weight of 5 to 12 mg. They are easily inserted inside the cul de sac of the eye without irritation. Depending on their chemical nature, they may gel in the presence of ocular fluid, or the tablet matrix dissolves, thus providing drug release. Levofloxacin is frequently preferred antibiotic drug for the treatment of bacterial conjunctivitis or keratitis and hence selected as the drug candidate to formulate ocular minitablets.

Advantages of ocular minitablets[(6)]

1. Increased bioavailability

2. Extended duration of action
3. Reduced number of instillation
4. Minimized systemic side effects
5. Accurate dosing
6. Increased shelf life with respect to aqueous solutions
7. Exclusion of preservatives, thus reducing the risk of sensitivity reactions
8. Conventional and well understood manufacturing techniques, low cost and predictable behavior

Table 1. Some Characteristics of Ocular Route of Drug Administration[(5)]

Route	Absorption Pattern	Special Utility	Limitations
Topical	Prompt, depending on formulation	Convenient, economical, relatively safe	Compliance , corneal and conjunctival toxicity, nasal mucosal toxicity, systemic side effects from nasolacrimal absorption
Subconjunctival, sub Tenon's, and retrobulbar injections	Prompt or sustained, depending on formulation	Anterior segment infections, posterior uveitis, cystoid macula edema	Local toxicity, tissue injury, globe perforation, optic curve trauma, central retinal artery and /or vein occlusion, direct retinal drug toxicity
Intraocular injections	Prompt	Anterior segment surgery	Corneal toxicity
Intravitreal injection or device	Absorption ircumvented, immediate local effect	Endophthalmitis, retinitis	Retinal toxicity

2. MATERIALS AND METHODS

2.1 Materials

HPMC K4M, Carbopol 971G, Levofloxacin, HPMC K100 M were obtained as a gift sample from Glaxo Smith Kline and all other chemicals and excipients used were of analytical grade.

2.2 Preparation of minitablets

The powder mixture HPMC K4M, Carbopol 971G, HPMC K100 M, Na CMC, Levofloxacin and magnesium stearate (w/w) was obtained by homogeneously mixing the different compounds. The minitablets (diameter 4 mm, weight 35 mg) were prepared by directly compressing the powder mixture manually. The ocular minitablets were sterilized by gamma radiation after packaging and the packages were exposed to a dose of 25 kGy (Dose rate: 10.5 kGy/hr). The total dose was given over 6 hrs. Table 2 explains the different batches of ocular minitablets.

Table 2. Composition of Different Batches

Ingredients	Batches (mg)		
	L9	L11	L12
Levofloxacin	9	9	9
HPMC K100 M	6	7	7
Carbopol 971G	1	1	1
Magnesium Stearate	1	1	1
HPMC K4M	12	7	7
MCC	6	10	7
Xanthan Gum		3	
Sodium CMC			3
Total	35	35	35

2.3 Physical parameters

The prepared minitablets were evaluated in terms of the test such as hardness, thickness and friability by using Monsanto hardness tester, Vernier caliper and digital friability tester respectively as per Indian Pharmacopeia 2007.

2.4 Drug Content[7]

10 tablets were crushed and power equivalent to 10 mg was weighed. Then it was dissolved in simulated tear fluid and volume was made up to 100 ml. from this 100 µg/ml stock solution 1ml aliquot was removed and diluted up to 10 ml. this sample was run in UV spectrum and absorbance was taken. Then drug content was calculated. This was done in triplicate manner.

2.5 Determination of bio adhesive strength:[7]

A modified balance method was adopted to measure the bioadhesion properties. A goat conjunctival membrane was dissected and placed in normal saline after being washed. The membrane, cut into 1 cm lengths was stuck to a moving platform by using glue. From each batch, minitablet was taken and applied on the lower surface of the upper polypropylene cylinder. The beaker containing mucosal tissue secured upon lower cylinder (E), was manipulated over the base of the balance so that, the mucosal tissue is exactly below the upper cylinder (D). The exposed part of the minitablet was wetted with a drop of simulated tear fluid, and then a weight of 10 gm was placed above the expanded cap, left for 10 minutes. After which the minitablet binds with mucin. The weight was removed. Then slowly and gradually weights were added on the right side pan till the gel separates from the mucosal surface/ membrane.

The weight required for complete detachment is noted (W1) (W1-5.25gm)) gives force required for detachment expressed in weight in grams. Procedure was repeated for two more times. Average was computed and recorded. The force of adhesion was calculated: force of adhesion (N) = (Bioadhesive strength ×9.81)/1000.

2.6 Swelling index

2.6.1 Preparation of simulated tear fluid

2.18 g of $NaHCO_3$, 6.78 g NaCl, 0.084 g $CaCl_2.2H_2O$ and 1.38 g of KCl was weighed accurately and dissolved in distilled water. Volume was made up to 1000 ml with distilled water pH of the solution was maintained at 7.4[8]. Three tablets were weighed individually (W_1) and immersed in a Petri plates containing simulated tear fluid (pH 7.4) for predetermined times (15min, 30 min,1, 2, 4, 8h). The tablets were removed at predetermined interval and wiped off with filter paper to remove excess water and weighed (W_2)[9]. The % swelling index was calculated by: % Swelling Index = [W_2- W_1] / W_2 x 100 Where W_1 is the initial weight of the tablet and W_2 is the weight of the tablet after the particular swelling time interval.

2.7 Isotonisity Evaluation[10]

The formulations were mixed with few drops of diluted blood on a slide. The diluted blood was prepared by using Grower's solution and Slide was observed under microscope at 45x magnification. The shape of blood cells were compared with standard marketed ophthalmic formulation.

2.8 In-vitro Drug Release Study

2.8.1 In vitro Diffusion study[11,12]

In-vitro release study of the formulated ophthalmic mintablet was carried out by using diffusion cell through egg membrane as a biological membrane. Diffusion cell with inner diameter 24mm was used for the study. the minitablet was placed in donor compartment and Freshly prepared 100 ml artificial tear fluid (sodium chloride 0.670g, sodium bicarbonate 0.200g, calcium chloride dehydrated 0.008g, purified water q.s 100ml.) in receptor compartment. Egg membranes were mounted in between donor and receptor compartment. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°C ± 0.5°C. 1 ml of sample is withdrawn from receiver compartment after 1, 2, 3, 4, 5, 6, 7 & 8 hrs. and same

volume of fresh medium is replaced. The withdrawn samples was diluted to 10ml in a volumetric flask with methanol and analyzed by UV spectrophotometer at 287.0 nm.

Figure 6.Laboratory designed diffusion cell A- Tube containing formulation, B- Egg membrane, and C- Beaker containing simulated tear fluid.

2.8.2 In vitro dissolution study

The rotating glass vial method [13]

This method was based on NF XIV guideline. Size of the vial was 1.5 cm diameter x 3.3 cm height. It was attached to the end of basket drive in off centered manner in the dissolution jar of USP apparatus type 2. The jar having adequate quantity of water was used as secondary water bath for the bottle. The mintablet was accurately weighed, and transferred to glass vial containing 5ml simulated tear fluid, closed with rubber cap placed, attached to the drive and positioned in the secondary water bath at 32 ± 0.50º C (eye surface temperature) and rotated separately at 25rpm.Samples measuring 1 ml each were withdrawn at 1hr, 4 hr, 8 hr and 12 hr. Thus, the total duration was aimed to be 12 hrs. Each aliquot withdrawal was followed by immediate replenishment with 1 ml of the medium at the same temperature. The samples were diluted to 100 dilution factor with simulated tear fluid. UV absorbance of these was measured at 287 nm on a Jasco V630 Spectrophotometer against an appropriate blank. The concentration of drug in the samples was calculated from the standard plots of the drug in the simulated tear fluid.

Figure 7.Dissolution apparatus

2.9 Antibacterial Activity[14]

An agar diffusion method was used for the determination of antifungal activity of formulations. Standard Petri dishes (9 cm diameter) containing medium to a depth of 0.5 cm were used. The sterility of the lots was controlled before use. Inoculum were prepared by suspending 1-2 colonies of *Staphylococcus aureus* from 24 hrs.Cultures in nutrient agar medium into tubes containing 10 ml of sterile saline. The tubes were diluted with saline. The inoculum (0.5 ml) was spread over the surface of agar and the plates were dried at 35°C for 15 min prior to placing the formulation. The bores of 0.5 cm diameter were prepared and samples of minitablet were added in the bores. After incubation at 35°C for 24 h, the zone of inhibition around the bores was measured.

3. RESULTS AND DISCUSSION

The prepared minitablets were yellowish colored, smooth in texture, uniform in appearance. The drug content of formulation was found to be uniform and within acceptable limits. Results of physical parameters were discussed in Table 3.

Table 3.Resultsof Physical Parameters

Formulation code	Hardness	Thickness (mm)	Weight variation	% riability	% Drug content
L9	3±0.170	2±0.1449	34.73±0.6396	0.57±0.011	98.13±0.4454
L11	3±0.070	2±0.1264	34.7±0.5832	0.56±0.015	98.26±0.5939
L12	3±0.124	2±0.1265	34.7±0.5349	0.27±0.577	101.7±0.6363

3.1 Bioadhesive strength

The detachment stress of formulation is shown in Table 4.

Table 4.Detachment stress of formulation

Sr. no.	Formulation code	Bioadhesive strength (Newton)
1	L9	0.3310±0
2	L11	1.0733±0.0736
3	L12	0.9883±0

Bioadhesive force means the force with which minitablet bind to ocular mucosa. Greater bioadhesion is indicative of prolonged residence time of a minitablets and thus prevents its drainage from cul-de-sac. The Bioadhesion force increased significantly as the concentration of bioadhesion polymers increased.The Detachment Stress was determined for ocular minitablet. **3.2 Swelling index** Results of swelling index study were given in table 5

Table 5.Swelling Index

Time in hrs.	Batch		
	L9	L11	L12
15 min	0.1238±0.032	0.2095±0.059	0.3523±0.059
30 min	0.4380±0.016	0.4380±0.043	0.5809±0.049
1 hr	0.6857±0.028	0.7238±0.043	0.8666±0.071
2 hr	0.8476±0.043	0.9047±0.059	1.1523±0.072
3 hr	1.2190±0.071	1.2476±0.1	1.3619±0.070

3.3 Isotonisity

The shape of blood cells, blood cells with Levofloxacin formulation and blood cells with. Levofloxacin eye drop as a marketed formulation are shown in Fig.8a, Fig 8b, and Fig. 8 c Isotonisity testing of formulations exhibited no change in the shape of blood cells. The blood cell size was found in 7-8µm range which reveals the isotonic nature of the formulations as compared with standard marketed ophthalmic formulation. This indicates the maintenance of tonicity in prepared formulations. Isotonisity was maintained to prevent tissue damage of eye.

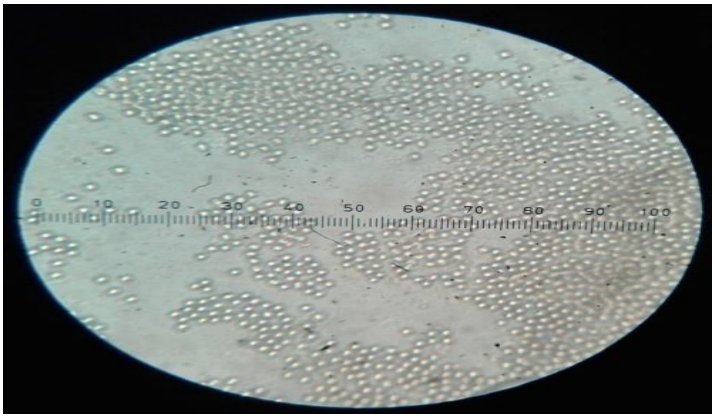

Figure 8a. Blood cells

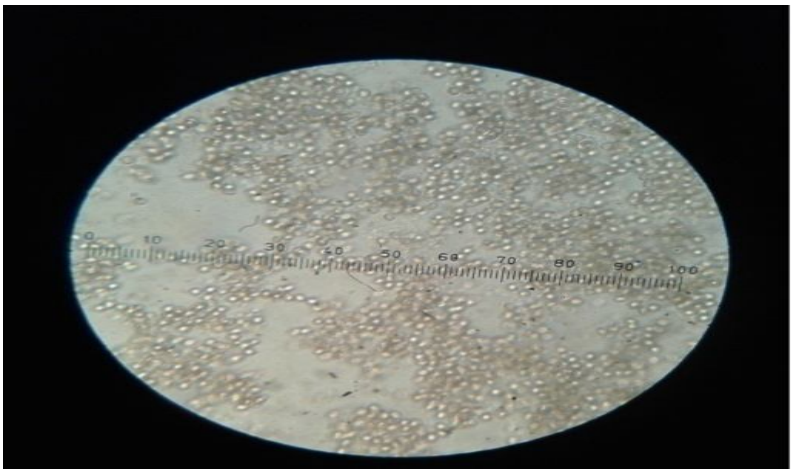

Figure 8b. Blood cells with formulation

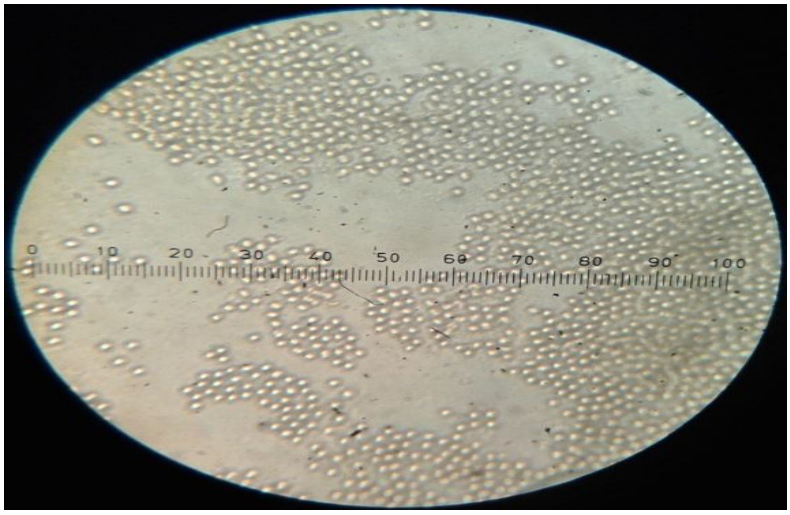

Figure 8c. Blood cells with Levofloxacin marketed formulation

3.4 In vitro drug release study

The *In-vitro* drug release study of formulation (Table 6). Drug release study was also carried out by rotating glass vial method. The results of the dissolution study were given in table 6

Table 6. Drug Release by Dissolution Study

Sr.No.	Time interval (hrs.)	Batches % CDR		
		L9	L11	L12
1	1	17.76395	18.40332	14.73129
2	4	35.40349	31.69518	38.62278
3	8	57.67064	54.74339	59.49542
4	12	88.78694	92.338	94.31139

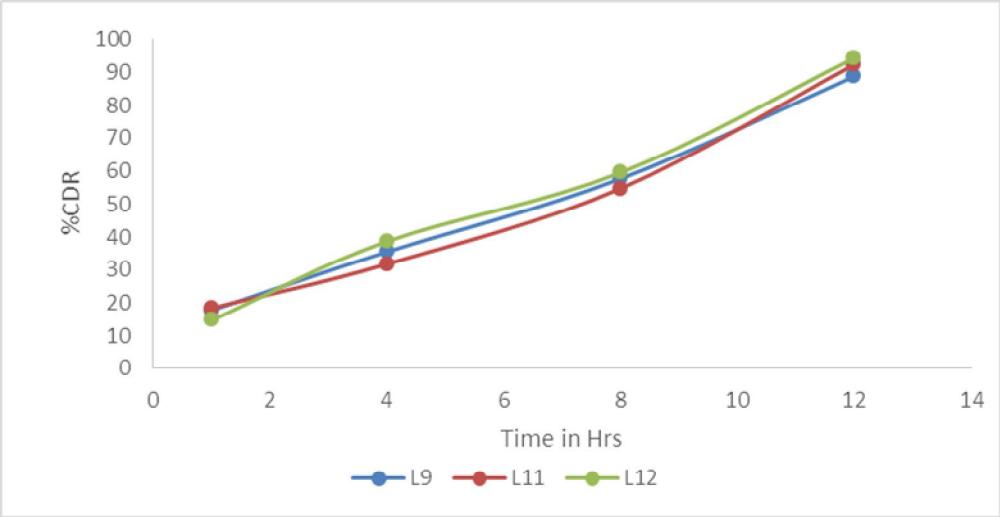

Figure 9.In-vitro Drug Release Profile

From the in vitro dissolution and diffusion data the release of batch L12 found best hence batch L12 was selected.

3.5 Release Kinetics

In the present study, the drug release was analyzed by PCP Disso version software to study the kinetics of drug release mechanism. To analyze the mechanism of drug release from the tablet, data obtained from the drug release studies was subjected to different mathematical models (Zero order, First order, Matrix (Higuchi) and Korsmeyer's - Peppas). The correlation coefficient (r^2) was used as an indicator for the best fitting for each of the models. Table 7 shows the Kinetics treatment for the batch L12 formulations.

Table 7.Model fitting of Batch L12

Zero order	1st order	Matrix	Peppas		Hixon Crowel
			r^2	n-Value	
0.9955	0.9960	0.9543	0.9921	0.9334	0.9974

As the R value of batch L12 is near to 0.999 in zero order and also k value for the same is 8.6203 so it is best fitted for zero order model.

Table 8.Best Model fitting of batch L12

Sr. No.	Model Fitting	r^2 Value	K
1.	Zero order	0.9955	8.6203

Discussion: From the r^2 value it was concluded that the drug release profile of batch L12 of Levofloxacin followed zero order release pattern. if the exponent n = 0.5, then the drug release mechanism is Fickian diffusion, and if 0.5 <n < 1.0, then it is non-Flickian. The value of n in case of Levofloxacin minitablet was above 0.5, which indicats that the non-fickanian diffusion mechanism.

3.6 Antibacterial activity

The result of antibacterial activity of formulations (Table 9).

Table 9. Zone of inhibition

Sr. No.	Batch L12 code	*Staphylococcus aureus*	
		Zone of inhibition (mm)	% Efficacy
1	Standard	17-28	100
2	L9	21±3.60	75
3	L11	23±1.52	82.14
4	L12	24±3.51	85.71
5	Drug	23±3.51	82.14
6	Marketed	22±2.51	78.57

The standard value of Levofloxacin against *staphylococcus aureus* for zone of inhibition is 17-28 mm.Thestudy indicates that Levofloxacin retained its antifungal efficacy when formulated as an ocular minitablet and drug was active against selected strains of micro-organism. L12 formulation showed 24mm zone of inhibition. A result obtained from antifungal activity of L12 formulation resembles to release profile of drug which indicates the dependency of antibacterial activity with drug release from formulation.

4. CONCLUSION

The dissolution studies of different batches showed that batch L12 showed the maximum drug release. Also antibacterial activity results showed that batch L12 was best when compared with standard drug and marketed formulation. These findings suggest the potential of Levofloxacin ocular minitablet as an excellent delivery system for the treatment of bacterial keratitis and conjunctivitis.

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Conflict of interest statement

The authors report no conflicts of interest.The authors aloneare responsible for the content and writing of the paper.

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