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Research Article

Novel Formulation Development and Evaluation of Nanoparticles Based *in Situ* Gelling System for The Ophthalmic Delivery of Ciprofloxacin Hydrochloride

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ABSTRACT

Ciprofloxacin hydrochloride loaded Eudragit RS100 nanoparticles were prepared by using w/o/w emulsification (multiple emulsification) solvent evaporation followed by drying of nanoparticles at 50°C. The nanoparticles were further incorporated into the pH-triggered *in situ* gel forming system which was prepared using Carbopol 940 in combination with HPMC as viscosifying agent. The developed nanoparticles was evaluated for particle size, zeta potential value and loading efficiency; nanoparticle incorporated *in situ* gelling system was evaluated for pH, clarity, gelling strength, rheological studies, *in-vitro* release studies and *ex-vivo* precorneal permeation studies. The nanopaticle showed the mean particle size varying between 263.5nm - 325.9 nm with the mean zeta potential value of -5.91 mV to -8.13 mV and drug loading capacity varied individually between 72.50% to 98.70% w/w. The formulation was clear with no suspended particles, showed good gelling properties. The gelling was quick and remained for longer time period. The developed formulation was therapeutically efficacious, stable and non-irritant. It provided the sustained release of drug over a period of 8-10 hours.

Keywords: Ciprofloxacin hydrochloride, *in situ* gel, nanoparticles, precorneal permeation, *in-vitro, ex-vivo.*

INTRODUCTION

Eye drops are conventional ophthalmic drug delivery system often result in poor bioavailability and therapeutic response, because of the high tear fluid turnover, rapid precorneal elimination of drug[1], non-productive absorption[2] and limited permeability of hydrophilic drugs through the corneal epithelium and tear film via paracellular route, which are lipophilic in nature [3,4]. As a result, frequent dosing is usually needed in order to avoid the rapid dilution[5]. This in turn leads to extensive systemic absorption and result in unwanted side effects[6,7]. These problems can be overcome by using nanopaticle based *in situ* gelling system for the ophthalmic delivery system prepared from polymers that exhibit reversible phase transition (sol-gel-sol) and pseudo plastic behaviour to minimize interference with blinking of eye[2,8]. Gelation can be triggered by temperature, pH, ions, solvent induction and may be UV light induction. But three major methods have been employed to cause phase transition on the surface: change in temperature, pH, and electrolyte composition[9,10].

While ophthalmic use of dispersed system of nanoparticles overcomes the problem of low bioavailability of the topical conventional eye drops[11]. Biodegradable nanoparticles have the advantage of controlled release of drug incorporated to obtain the required tear level and their therapeutic effects and possibly the mucoadhesion to prolong the residence time of the particles in the precorneal area or of endocytosis through the corneal epithelium, depending upon the particle size and size distribution[7,12,13].

Ciprofloxacin hydrochloride Fig. (1), chemically 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid hydrochloride monohydrate[14] is a powerful 4th generation fluoroquinolone antibiotic, useful in the treatment of infections of outer eye such as bacterial conjunctivitis and keratitis caused by gram positive and gram negative ocular pathogens such as *Pseudomonas aeruginosa* and *staphylococcus aureus*[15,16]. The Fluoroquinolone work by two Mechanisms:

Inhibition of enzyme bacterial DNA Gyrase

Inhibition of enzyme Topoisomerase IV[17]

The pH-triggered *in situ* gelling system as well as drug

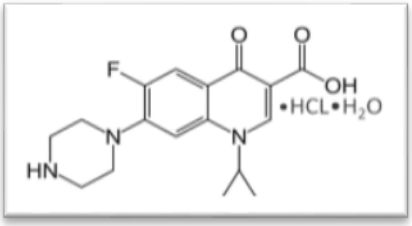

Figure 1: Chemical structure of Ciprofloxacin hydrochloride[15]

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Table 1: Different batches of nanoparticles incorporated *in situ* gelling system

Formulation	HPMC grade	Concentration (% w/v) HPMC	Carbopol 940
F1	K100M	0.25	0.1
F2	K100M	0.50	0.1
F3	K50M	0.25	0.1
F4	K50M	0.50	0.1
F5	K15M	0.25	0.1
F6	K15M	0.50	0.1

loaded nanoparticles of 4th generation fluoroquinolone (Ciprofloxacin hydrochloride) has been developed for external infection of eyes[18]. Carbopol 940 and HPMC were used in combination for the preparation of pH-triggered *in situ* gelling system[6,19]. While Eudragit RS100 polymer have been proposed as ocular delivery system with prolonged release and improved ocular bioavailability[20,21].

In this study we prepared Ciprofloxacin hydrochloride loaded Eudragit RS100 nanoparticles using w/o/w emulsification method[22] which was further incorporated into *in situ* gelling system prepared using carbopol 940 in combination with HPMC for the ophthalmic delivery of Ciprofloxacin hydrochloride[19] against bacterial infections such as keratitis, conjunctivitis etc. caused by *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*[17]. Physicochemical properties of Ciprofloxacin hydrochloride-loaded nanoparticles and nanopaticle incorporated *in situ* gelling system were investigated.

MATERIALS AND METHODS

Materials

Analytical pure drug Ciprofloxacin hydrochloride was purchased from Jackson Laboratories, (Amritsar, India). Eudragit RS100 was purchased from Evonik Rohm GmbH., (Darmstadt, Germany). Carbopol 940 and PVA (MW > 1, 00,000) were purchased from SD fine-chem Ltd., (Mumbai, India). While HPMC K100M, HPMC K50M, HPMC K15M, Benzlkonium chloride and Tween 80 were purchased from Loba Chemie pvt. Ltd., (Mumbai, India). All the chemicals used were of analytical grade.

Preparation of Ciprofloxacin hydrochloride-loaded Eudragit RS100 nanoparticles

Preparation of Ciprofloxacin hydrochloride aqueous solution

A solution of 2.5% w/v of Ciprofloxacin hydrochloride was prepared by dissolving the drug in distilled water. The solution was sonicated for 2 minutes using Ultrasonic cleaner-012 (Loba science, Mumbai, India).

Preparation of nanoparticles

The nanoparticles were prepared by w/o/w emulsification solvent evaporation followed by high speed stirring. Accurately measured 25 ml of Ciprofloxacin hydrochloride aqueous solution and it was emulsified by means of sonication for 2 minutes in an organic polymer phase i.e. Eudragit RS100 (0.25-2.0% w/v) in 250 ml of acetone. The resulting w/o emulsion was dispersed in aqueous stabilizer phase i.e. PVA (0.1-0.5% w/v) in distilled water and sonicated for 2 minutes to obtain a multiple w/o/w emulsion, which was stirred at 6000 rpm for 6 hours using mechanical stirrer (RQ 124-A, Remi electrotechnik ltd, Vasai, India). The emulsion was then diluted with 100 ml 0.3% w/v aqueous stabilizer (PVA) solution in order to minimize coalescence of the emulsion and aggregation of the particles formed. The organic phase was allowed to evaporate at room temperature under agitation with magnetic stirrer (REMI 2MLH, Remi electrotechnik ltd, vasai, India) for 4 hours. Consequently the polymer, insoluble in the water phase, precipitated as solid particles. The resulting nanosuspension was dried at 50°C in hot air over (Navyug scientific ltd, Ambala, India.

Evaluation of the Nanoparticles

Nanopaticle Size and Zeta potential analysis

The mean particle size Z_{avg} and zeta potential of the nanoparticles was determined by Photon Correlation Spectroscopy (PCS) and Electrophoretic Light Scattering (ELS) respectively by using Zetasizer 6.30 (Malvern Instruments, Malvern, France). A portion of freshly prepared suspension was diluted hundred times with purified water. The Z_{avg} and zeta potential of each sample was determined four times. Afterwards, the average values were used in optimal surface response design for plotting the response surfaces.

Determination of drug content and drug loading capacity

To determine the drug content, 50 mg of nanoparticles, were dispersed in 10 ml of purified water and gently sonicated for 10 minutes. The sample was centrifuged at 3000 rpm for 3 hours (R-8C, Remi motors Ltd, Mumbai, India) and the Ciprofloxacin hydrochloride concentration in the supernatant was determined at 272.8 nm using (UV-spectrophotometer 1800, Shimadzu Co. Ltd., Japan). Drug content and loading efficiency were determined by using following formula:

$$\text{Drug Content} = \{\frac{\text{Drug weight in nanoparticles}}{\text{total weight of nanoparticles}}\}\text{x}100$$

$$\text{Loading efficiency} = \{\frac{\text{Drug remained in the nanoparticles}}{\text{feeding weight of drug}}\}\text{x}100$$

Preparation of Ciprofloxacin hydrochloride-loaded nanopaticle incorporated in situ gelling system

Preparation of Phosphate buffer (pH 6.4)

Phosphate buffer (pH 6.4) was prepared using 0.2M potassium dihydrogen phosphate solution (25 ml) and 0.2M sodium hydroxide solution (5.8 ml) and final volume was adjusted to 100 ml with distilled water and pH was adjusted to 6.4 using 0.2M sodium hydroxide solution.

Preparation of Polymer solution

Hydroxy Propyl Methyl Cellulose (0.25% w/v and 0.5% w/v) was added into 75 ml of the phosphate buffer (pH 6.4) and allowed to hydrate with stirring on magnetic stirrer. Carbopol 940 (0.1% w/v) was sprinkled over the solution and allowed to hydrate overnight with continuous stirring on magnetic stirrer. Tween 80 (0.5% v/v) was added whilst stirring.

Preparation of Ciprofloxacin hydrochloride-loaded nanopaticle solution

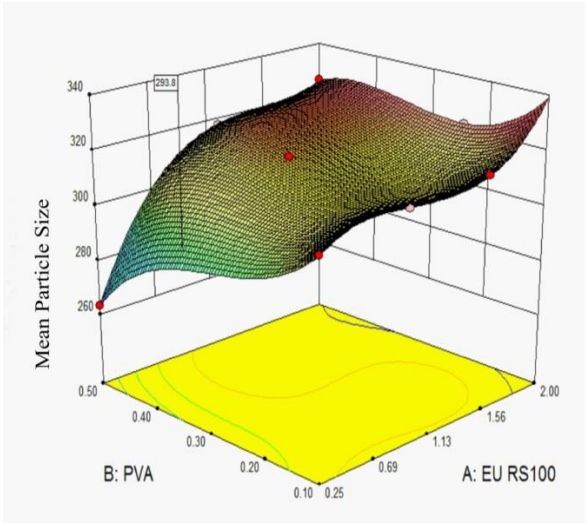

Figure 2: Three dimensional surface plot for effect of polymer (Eudragit RS100) and stabilizer (PVA) concentration on mean particles sizes and zeta potential of prepared nanoparticles.

Table 2: Drug content and loading efficiency of the different batches

Batch	Weight Ratio (w:w) Eudragit RS100 + PVA : Ciprofloxacin hydrochloride	Drug Content in 50 mg of nanoparticles		Loading Efficiency (% w/w)
		Theoretical (x) (%)	Experimental (y) (%)	
1	4.075:0.650	15.95	12.010	75.29
2	4.175:0.650	15.568	12.279	78.87
3	1.875:0.650	34.667	32.009	92.33
4	5.100:0.650	12.740	9.723	76.31
5	5.575:0.650	11.650	8.996	77.21
6	6.250:0.650	10.400	7.540	72.50
7	2.050:0.650	31.700	31.289	98.70
8	2.500:0.650	26.000	24.583	94.55
9	3.325:0.650	19.540	18.913	96.79
10	1.750:0.650	37.140	36.574	98.47
11	0.875:0.650	74.280	70.751	96.94

Ciprofloxacin hydrochloride-loaded nanoparticles were dispersed in 20 ml of phosphate buffer solution pH 6.4. Benzlkonium chloride solution (0.02% v/v) was then added. The solution was filtered through 0.2 µm Whatman filter paper.

Preparation of in situ gelling system

The Ciprofloxacin hydrochloride-loaded nanopaticle solution was then poured into the polymer solution under constant stirring until a uniform solution was obtained. Phosphate buffer (pH 6.4) was then added to make up the final volume to 100 ml and pH of the final solution was adjusted to 6.4 using 0.2M sodium hydroxide. Different batches of nanoparticles incorporated *in situ* gelling system are shown in table 1.

Evaluation of Ciprofloxacin hydrochloride-loaded nanopaticle incorporated in situ gelling system

Evaluation of physical parameters

The formulated Ciprofloxacin hydrochloride-loaded nanopaticle incorporated *in situ* gelling system was evaluated for clarity, suspended particles and pH of the solution. The clarity of the formulation was determined by visual investigation of the formulation with naked eye. The suspended particles in formulation were checked against black and white background. While the pH of the formulation was checked in solution form and after mixing it with simulated tear fluid using auto digital pH meter (LT-11, Labtronics, panchkula, Haryana).

Rheological studies

The developed formulation was poured into the small sample adaptor of the Brookfield synchrolectric viscometer (DV-E viscometer, USA) and the angular velocity increased gradually from 0.5 to 50 rpm. The average of the two readings was used to calculate the viscosity. The formulation was then poured into an ointment jar and the pH raised to 7.4 by adding 0.5M NaOH. The rheology of the resultant gel was also studied.

Gelling capacity

The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of artificial tear fluid freshly prepared, equilibrated at 37°C and pH 7.4. we observed the gel formulation visually and noting the time for gelation and the time taken for the gel formed to dissolve.

Percentage drug content

The percentage drug content was determined by diluting the 1 ml of the formulation to 100 ml of phosphate buffer

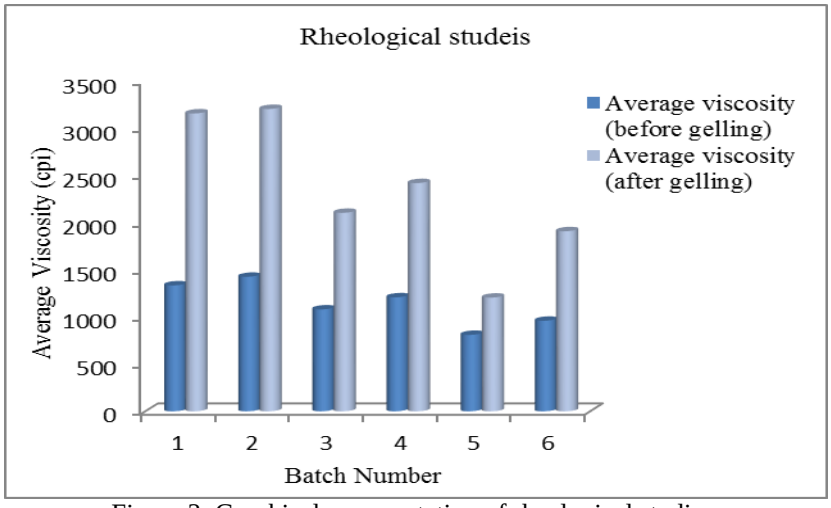

Figure 3: Graphical representation of rheological studies.

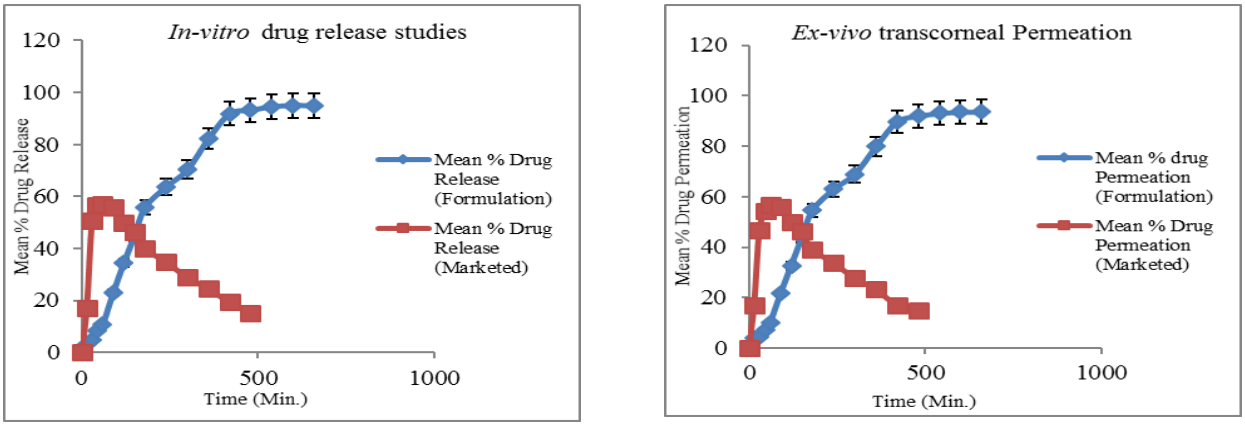

Figure 4: Comparative study of Marketed formulation and prepared nanopaticle incorporated pH-triggered *in situ* gel forming system for *in-vitro* drug release and *ex-vivo* transcorneal permeation studies respectively.

(pH 6.4) and analysed using (UV-spectrophotometer 1800, Shimadzu Co. Ltd., Japan) at 272.8 nm. The concentration of the drug then calculated from the calibration curve using regression equation method.

In-vitro drug release studies and Ex-vivo transcorneal permeation studies

The *in-vitro* release studies and *ex-vivo* transcorneal permeation studies of Ciprofloxacin hydrochloride from the formulations was studies through cellophane membrane and goat cornea respectively using a modified Franz diffusion apparatus. The freshly prepared simulated tear fluid (pH 7.4) was used as a diffusion medium. Semi-permeable membrane previously soaked in the diffusion medium for overnight, while Fresh whole eyeballs of goats were obtained from a local butcher's shop and transported to the laboratory in cold condition in normal saline. Corneas were then carefully removed along with 5 to 6 mm of surrounding scleral tissue and stored in freshly prepared artificial tear solution pH 7.4 were fixed in-between the donner and receiver compartment of the modified Franz diffusion apparatus. 1ml of the formulation was mixed with 5 ml of the simulated tear fluid and poured into the donner compartment. Receiver compartment was maintained at 37±2°C with a stirring rate of 50 rpm using magnetic stirrer. About 1 ml of the perfusate was withdrawn at a timer interval of 15 min, 30 min, 45 min, 60 min, 120 min, 180 min, 240 min, 300 min, 360 min, and 420 min, 480 min, 540 min, 600 min, 660 min and replaced with an equal volume of fresh diffusion medium. The aliquot was diluted with the diffusion medium and analysed at 272.8 nm using (UV-spectrophotometer 1800, Shimadzu Co. Ltd., Japan). 1ml of the pure drug solution (0.5% w/v in distilled water) and 1 ml of marketed product (CIPLOX) were evaluated in a similar manner.

RESULTS AND DISCUSSION

Evaluation of Nanoparticles

Particle Size and zeta potential

The effect of stirring, polymer and stabilizer concentration on the nanoparticles size is visible.

The concentration of polymer can stabilizer greatly affects the size of the drug nanoparticles. Higher concentrations of polymer leads to bigger particle size and higher concentration of stabilizer leads to smaller particles size with more stability. The particle sizes of the samples are graphically represented in Fig. (2). The particle size ranging from 263.5-325.9 nm was measured. It was found that the other variable factors did not have a significant (*P value <0.0001*) influence on the size of the nanoparticles.

The value of the zeta potential of all particles was approximately the same and varied between the individual extreme values of -5.91 and -8.20 mV with no significant (*P value < 0.0007)* as shown in Fig. (2).

Determination of drug content and drug loading capacity

The loading efficiency of all the batches was good. It varied individually between 72.50% -98.70% w/w, as shown in table 2. Which shows that, Eudragit RS100 having greater drug entrapment. The loading efficiency indicates that higher the feeding amount of drug and polymer increased the drug content and loading efficiency.

Evaluation of Ciprofloxacin nanopaticle incorporated in situ gelling system

Physical parameters evaluation

All the formulations were clear light yellow in colour with no suspended particles. F1, F3, F5, F6 were translucent while F2 and F4 were transparent and the pH was near around 6.0 – 6.5 for all the formulations before instillation, while pH raised to 7.4 after mixing it with simulated tear fluid.

Rheological studies

Rheological evaluation of all the formulation exhibited Newtonian flow before gelling and exhibited pseudoplastic flow after gelling. There was increase in the viscosity after gelling. Additionally, the gel formed *in situ* should maintain its integrity without dissolving or eroding for a prolonged period of time. Results are graphically represented in Fig. (3).

Gelling capacity

Formulations prepared from Carbopol 940 (0.1% w/v) and HPMC K100M, HPMC K50M, HPMC K15M (0.25 % w/v and 0.5% w/v) showed better gelling capacity. Formulation F1, F2 showed (+++ gelation), gelation immediate, remains for 7-9 hours, F3, F4, F6 showed (++ gelation), gelation immediate, remains for 2-3 hours while F5 showed (+ gelation), gel after few minutes, dissolves rapidly.

Percentage drug content

The drug content of all the formulations was within the range of 99.37±0.10% - 95.16±0.27% w/w which was showing that the formulation had the better drug loading capacity.

In-vitro drug release studies and Ex-vivo transcorneal permeation studies

The comparative *in-vitro* drug release studies and *ex-vivo* transcorneal permeation studies were carried out for Ciprofloxacin hydrochloride marketed formulation (CIPLOX®) and prepared nanopaticle incorporated pH-triggered *in situ* gel forming systems. The results are shown in Fig. (4).

CONCLUSION

The formulations were therapeutically efficacious. The developed formulation is a viable alternative to the conventional eye drops by virtue of its ability to enhanced bioavailability through its longer precorneal residence time, greater permeability through the tear film and corneal layers and ability to sustain drug release. Also important is the ease of administration and decreased frequency of administration resulting in better patient acceptance.

REFERENCES

1. Srividya, B.; Cardoza, R.M.; Amin, P.D. Sustained ophthalmic delivery of ofloxacin from a pH triggered *in situ* gelling system. *J. Control Release,* 2001, 73, 205–211.
2. Budai, L.; hajdu, M.; Budai, M.; Grof, P.; Beni, S.; Noszal, B.; Klebovich, I.; Antal, I. Gels and liposomes in optimized ocular drug delivery: studies on ciprofloxacin formulations. *Int. J. Pharm.,* 2007, 343, 34-40.
3. Liu, Z.; Li, J.; Nie, S.; Liu, H.; Ding, P.; Pan, W. Study of an alginate/HPMC-based *in situ* gelling ophthalmic delivery system for Gatifloxacin. *Int. J. Pharm.,* 2006, 315, 12-17.
4. Araujo, J.; Gonzalez, E.; Egea, M.A.; Garcia, M.L.; Souto, E.B. Nanomedicines for ocular NSAIDs: safety on drug delivery. *Nanomedicine,* 2009, 5, 394-401.
5. Greateri, T.; Gelfuso. G.M.; Rocha, E.M.; Sarmento, V.H.; Freitas, O.D.; Lopez, R.F.V. A Poloxamer/Chitosan *in situ* forming gel with prolonged retention time for ocular delivery. *Eur. J. Pharm. Biopharm.,* 2010, 75, 186-193.
6. Mohanambal, E.; Arun, K.; Abdul, H.S.A. Formulation and Evaluation of pH-triggered *in situ* Gelling System of Levofloxacin. *Indian J. Pharma. Edu. Res.,* 2011, 45, 58-64.
7. Zimmer, A.; Kreuter, J. Microsphere and Nanoparticles used in ocular delivery systems. *Adv. Drug Deliv. Rev.,* 1995, 16, 61-73.
8. Bourlais, C.L.; Acer, L.; Zia, H.; Sado, P.A.; Needham, T.; Leverge, R. Ophthalmic drug delivery systems-recent advances. *Prog. Retin. Eye Res.,* 1998, 17(1), 33-58.
9. Rajoria, G.; Gupta, A. *In situ* gelling system: a novel approach for ocular drug delivery. *American J. pharmtech res.,* 2012, 2(4), 24-53.
10. Hajare, A.A.; Mali, M.N. *In situ* gel-forming systems for sustained ocular drug delivery. *Eur. Indust. pharmacy,* 2010, 5, 17-20.
11. Lamprecht, A.; Ubrich, N.; Perez, M.H.; Lehr, C.M.; Hoffman, M.; Maincent, P. Biodegradable monodispersed nanoparticles prepared by pressure homogenization-emulsification. *Int. J. Pharm.,* 1999, 184, 97-105.
12. Nagarwal, R.C.; Kantm S.; Singh, P.N.; Maiti, P.; Pandit, J.K. Polymeric nano-particulate system: A potential approach for ocular drug delivery. *J. Control Release,* 2009, 136, 2–13.
13. Hans, M.L.; Lowman, A.M. Biodegradable nanoparticles for drug delivery and targeting. *Cur. Opinion Solid state Mate. Sci.,* 2002, 6, 319-327.
14. Singh, H.; Kapoor, V.K. Quinolones and urinary tract antiseptics. In: Singh, H.; Kapoor, V.K. Medicinal and Pharmaceutical Chemistry. Second Edition. Vallabh Parkshan, 2005; pp. 553-556.
15. Indian Pharmacopoeia 2010. Volume II, Government of India, ministry of health and family welfare. The

Indian Pharmacopoeia Commission, Ghaziabad, 320-321.

16. Blondeau, J.M. Fluoroquinolones: mechanism of action, classification and development of resistance. *Surv Ophthalmol.,* 2004, 40, S73-S78.
17. Tripathi, K.D. Quinolones. In: Tripathi, K.D. Essentials of medical pharmacology. Sixth edition. Jaypee brother's medical publisher's ltd, 2008; pp. 687-691.
18. Mandal, S.; Thimmasetty, M.K.M.J.; Prabhushankar, G.L.; Geetha, M.S. Formulation and evaluation of an *in situ* gel forming ophthalmic formulation of moxifloxacin hydrochloride. *Int. J. Pharm. Investig.*, 2012, 2, 78-82.
19. Mohan, E.C.; Kandukuri, J.M.; Allenki, V. Preparation and evaluation of *in situ* gels for the ocular delivery. *J. Pharm. Res.,* 2009, 2(6), 1089-1094.
20. Dillen, K.; Vandervoort, J.; Mooter, G.V.D.; Ludwig, A. Evaluation of ciprofloxacin-loaded Eudragit® RS100 or RL100/PLGA nanoparticles. *Int. J. Pharm.*, 2006, 314, 72-82.
21. Gandomi, N.; Aboutaleb, E.; Noori, M.; Atyabi, F.; Fazeli, M.R.; Farbod, E.; Jamalifar, H.; Dinarvand, R. Solid lipid nanoparticles of Ciprofloxacin hydrochloride with enhanced antibacterial. *J. nanosci. letters*, 2011, 2(21), 1-7.
22. Singh, K. P.; Verma, S. Novel Polymeric in Situ Gel Forming System for Ophthalmic Drug Delivery. *Int. J. Drug Delivery Tech., 2014; 4(1); 1-13*