https://doi.org/ 10.33472/AFJBS.6.Si2.2024.2750-2763

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Formulation and Evaluation of Mucoadhesive Nanoparticles of Moxifloxacin as Ocular Drug Delivery System

Ankit[1], Pawan Jalwal[1], Shailja[1], Arun Kumar[2], Anshika Saini[1], Deepak[1]

[1]Faculty of Pharmaceutical Sciences, Baba Mastnath University, Rohtak.

[2]Department of Pharmaceutical Sciences, Gurugram University, Gurugram.

Corresponding Author

Pawan Jalwal

Email: pawan_jalwal@rediffmail.com

Article History

Volume 6,Issue Si2, 2024

Received:29 Mar 2024

Accepted : 30 Apr 2024

doi: 10.33472/AFJBS.6.Si2.2024.2750-2763

Abstract

The eye is a paired organ, the organ of vision. The eye is made up of various components, which enable it to receive light stimuli from the environment, and deliver these stimuli to the brain in the form of an electrical signal. The aim of this research was to develop formulation of the mucoadhesive nanoparticles of Moxifloxacin (BCS Class I drug) to increase residence time and bioavailability for the effective treatment of various ocular disorders like Glaucoma, conjunctivitis, corneal ulcer, trauma etc. The 12 trials batches with different ratios of excipients were taken, out of these three batches were blanks and nine were formulated with drug and best formulations were selected. The PDI of the formulations was varied from 0.137 to 0.512. Particle size of formulation varies from 183.7 nm to 962.1 nm. Zeta potential on the particles determines their physical stability (high zeta potential leads to more stable the colloid particles). The zeta potential of the formulations was varied from 14.2 to 27.5.Entrapment Efficiency of all formulation was found to be in the range of 39.80 to 94.24. The percent loading capacity of all formulation were found to be in the range of 3.32 to 7.87. Optimized formulation (F2)&marketedeyedropsofMoxifloxacinHClwereevaluatedforantimicrobial activity by cup-plate method. Formulation (F2) gave a clear zone of inhibition comparable with the zone of inhibition given by marketed eye drops. Results showed that formulation has better antimicrobial efficacy compared with the marketed eye drops.

Keywords: Ocular Drug Delivery, Nanoparticles, Moxifloxacin

Introduction

The eye is a paired organ, the organ of vision. The eye is made up of various components, which enable it to receive light stimuli from the environment, and deliver these stimuli to the brain in the form of an electrical signal. Vision involves all components of the eye. The dimensions of the eye are reasonably constant, varying among normal individuals by only a millimetre or two; the sagittal (vertical) diameter is about 24 mm (about one inch) and is usually less than the transverse diameter. [1] At birth the sagittal diameter is about 16 to 17 mm (about 0.65 inch); it increases rapidly to about 22.5 to 23 mm (about 0.89 inch) by the age of three years; between three and 13 the globe attains its full size. The weight is about 7.5 grams (0.25 ounce) and its volume 6.5 mm (0.4 cubic inch).

Ocular Drug Delivery System

Eye is most interesting organ due to its drug disposition characteristics. Topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy. [2] Drugs are commonly applied to the eye for a localized action, on the surface, or in the interior of the eye. A major problem in ocular therapeutics is the attainment of an optimal drug concentration at the site of action. Poor bioavailability of drugs from ocular dosage forms is mainly due to the precorneal loss factors which include tear dynamics, non-productive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the corneal epithelial membrane. Due to these physiological and anatomical constraints only a small fraction of the drug, effectively1% or even less of the instilled dose, is ocularly absorbed. So far, attempts have been made to improve ocular drug bioavailability by extending drug residence time in the conjunctival sac and improving drug penetration across the cornea, the major pathway of drug entry into the internal eye. [3]

The specific aim of designing a therapeutic system is to achieve an optimal concentration of a drug at the active site for the appropriate duration. Ocular disposition and elimination of a therapeutic agent is dependent upon its physicochemical properties as well as the relevant ocular anatomy and physiology. A successful design of a drug delivery system requires an integrated knowledge of the drug molecule and the constraints offered by the ocular route of administration. [4]

Nanoparticulate Drug Delivery System

With the intention of enhancing the medicinal and therapeutic characteristics of medications, drug delivery systems have been developed. Particulate dispersions or solid particles with a size between 10 and 1000 nm are referred to as nanoparticles. The medication is dissolved, trapped, or joined to a nanoparticle matrix. They frequently act as a sort of repository for the medications, storing them there until the proper moment to release them. As a result, they have an impact on the body's pharmacokinetics and drug distribution processes [5]. The goal of drug entrapment in nanoparticles is either improved delivery to or absorption by target cells or a decrease in the toxicity of the free drug to organs other than the target cells. [6] The therapeutic index will rise in both cases, with the difference between doses producing therapeutic efficacy (such as tumor cell death) and toxicity to other organ systems [7, 8]. This calls for the development of long-lasting and target-specific nanoparticles.

Materials and Methods

Materials

Moxifloxacin was obtained from Sigma. Chitosan, Tri-polyphosphate, Sodium Hydroxide were purchased from CDH Chemicals, Delhi. All other reagents used were of analytical grade.

Methods

Preparation of Nanoparticles

Chitosan solution (CS) was prepared by dissolving Chitosan in 1% (w/v) acetic acid solution under blending for the time being at room temperature. The Chitosan Solution was diluted with deionized water to produce different concentrations (0.1, 0.2, 0.3 %). CS/TPP nanoparticles were prepared as indicated to the ionotropic gelation process. In brief, TPP aqueous solution (0.1, 0.2, 0.3%) was added drop wise to the CS solution & stirred (1000 rpm) for 2 hr. at room temperature, to obtain blank nanoparticles. For preparation of moxifloxacin-loaded CS-TPP nanoparticles, the moxifloxacin solution 0.5% was added gradually to CS solution with mild stirring (1000 rpm) at room temperature and then TPP solution was added drop wise to the mixture with gentle stirring (1000 rpm) for 2 hr. Now 5% mannitol (cryoprotectant) is added to the prepared nanoparticles & the resultant solution is lyophilized. [9, 10]

Table 1: Composition of the Formulations

Formulation	Moxifloxacin (%)	Chitosan (%)	TPP (%)	Ratio (Chitosan : TPP)
B1	-	0.1	0.1	1:1
B2	-	0.2	0.2	1:1
B3	-	0.3	0.3	1:1
F1	0.5	0.1	0.1	1:1
F2	0.5	0.1	0.2	1:2
F3	0.5	0.1	0.3	1:3
F4	0.5	0.2	0.1	2:1
F5	0.5	0.2	0.2	2:2
F6	0.5	0.2	0.3	2:3
F7	0.5	0.3	0.1	3:1
F8	0.5	0.3	0.2	3:2
F9	0.5	0.3	0.3	3:3

Where,

B = Blank, F= Formulation

Evaluation of Nanoparticles

Appearance and Clarity

All the developed formulations were observed carefully for colour and presence of suspended particulate matter if any. The clarity of solutions was further assessed by observing them against a dark and white background. Formulations were graded as follows: (-) turbid, (+) slightly turbid, (++) clear solution, (+++) clear and transparent.

pH

The pH of ophthalmic formulation should be such that the formulation will remain stable at that pH and at the same time there would be no irritation to the patient upon instillation. Ophthalmic solutions should be formulated in a pH range of 6.5 to 8.5. pH of formulation was tested by the pH meter by dipping the pH meter in the beaker containing the formulation in which the electrode of pH meter comes in contact with the formulation solution. [11]

Entrapment Efficiency

To determine the entrapment of Moxifloxacin in nanoparticles, 1 ml of freshly prepared nanoparticle suspension was taken and diluted with appropriate STF (pH-7.4). Aliquots further subjected to cold centrifuge at ~4oC & 15,000 rpm using centrifuge for 30 minutes. The resulting solutions were analysed for Moxifloxacin content using a double beam UV spectrophotometer and % entrapment efficiency (% EE) was calculated using following equation.

$$\% \text{ EE} = \frac{\text{Total amount of drug} - \text{Free dissolved drug} \times 100}{\text{Total amount of drug}}$$

Loading Capacity

Loading capacity (%LC) helps to deal with nanoparticles after their separation from medium & to know their drug content

$$\% \text{ LC} = [\text{Entrapped drug} / \text{nanoparticles weight}] \times 100$$

Percentage Yield

The lyophilized nanoparticles from each formulation were weighed and the respective percentage yield was calculated using the following formula.

$$\text{Percentage Yield} = \frac{\text{Weight of Nanoparticles Obtained} \times 100}{\text{Weight of Drug, Polymer and Other Excipients Used}}$$

Polydispersity Index

PDI is an index of width or spread or variation within the particle size distribution. The usual range of PDI values is; <0.1 (monodisperse standard), 0.1 to <0.5 (nearly monodisperse), 0.5 to <1.0 (mid-range polydispersity), and >1.0 (highly polydisperse). [12, 13]

Particle Size Distribution & Zeta Potential

The particle size distribution & Zeta potential of the nanoparticles was analysed using a Dynamic Light Scattering (DLS) technique by Zetasizer without dilution at 25˚C. This technique assumes that all the particles are in Brownian motion in the solution and that all the particles are very small and spherical.

Transmission Electron Microscopy

Transmission electron microscopy is a technique in which a beam of electrons is transmitted through a specimen to form an image. The suspension is placed on a grid. An image is formed and the image is then magnified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor such as a scintillator attached to a charge-coupled device. [14]

Surface Morphology

Scanning Electron Microscope was used in order to examine the particle surface morphology and shape. A concentrated aqueous suspension was spread over a slab and

dried under a vacuum. The sample was shadowed in a cathodic evaporator with a gold layer 20 nm thick in an argon gas environment at 45 mA current for 5 seconds.

***In-vitro* Drug Release Studies**

In-vitro drug release of all medicated formulations was performed. The medium used was freshly prepared Phosphate buffer (pH 7.4). Previously soaked cellulose membrane was tied to one end of cylinder. Specific amount of the formulation was introduced into the tubes. Then the cylinders were attached to the metallic shaft and dipped in 100 ml of medium maintained at 34±1oC for the whole duration of study (24 hours). [15] The medium was rotated with magnetic bars at 50 rpm. At time intervals of 1hr, samples were withdrawn and replaced to maintain sink conditions.

Release Kinetics

In order to analyse the mechanism for the release and release rate kinetics of the dosage form, the in vitro release data obtained were fitted into a zero order, First order, Higuchi matrix, Korsmeyer-peppas, Hixson-Crowell models. [16,17] The best fit model was selected after analysing data to determine correlation coefficient (R^2) & release kinetics using various mathematical models:

Table 2: Different Models with Equations

Model	Equation
Zero order model	$Q = Q_0 + kt$
First order model	$Q = Q_0 \times e^{kt}$
Hixson-Crowell model	$Q^{1/3} = kt \times Q_0^{1/3}$
Korsmeyer-Peppas model	$Q = k \times t^n$

Where Q represents quantity of drug released in time t, Q_0 represents value of Q at zero-time, k represents the rate constant, n represents the diffusional exponent, a represents the time constant & b represents the shape constant parameter. The correlation coefficient (R^2) & the order of release pattern were calculated in each case. [18]

***Ex-vivo* Study**

Trans-corneal permeation potential of Moxifloxacin through CS-TPP nanoparticulate system was evaluated by using excised goat cornea with slight modification as reported. Cornea was isolated from goat eyes procured from freshly slaughtered animals at a local abattoir. The study was carried out in a Franz diffusion chamber. The excised goat cornea was mounted between donor & receiver compartments facing epithelial surface to donor compartment of the Franz diffusion cell. The donor compartment was filled with 6 mg of lyophilized prepared formulation that contains 0.5% of the drug content suspended in 1 ml STF. The receiver compartment was filled with freshly prepared STF. Study was carried out at 32±0.5ºC. Periodically, samples were collected for up to 4 hr. & subjected to quantification of Moxifloxacin by UV-VIS spectrophotometer. [19,20]

Microbiological Efficacy

The microbiological studies were carried out to ascertain the antimicrobial activity of the prepared formulations and to compare with the marketed eye drop, against *P. aeruginosa* (ATCC 6580) and *S. aureus* (NCTC 6749). A subculture of each organism was prepared by

transferring a loop full of each organism from laboratory-maintained cultures into 100 ml of sterilized nutrient broth and incubated for 24 h at 37 °C. Müller-Hinton-Agar medium was inoculated with the subculture (20 ml subculture/100 ml of Müller-Hinton-Agar), and 40 ml of the inoculated medium was transferred to each petri plate and allowed to solidify. Three wells were prepared aseptically in each plate with the help of a stainless-steel borer (8 mm diameter) so that the wells were separated equally from each other. The weighed quantities of all formulations were taken and suspended separately in normal saline solution (0.5% w/v) prior to the transfer into wells. Then 100 µl of each of the test solutions, as well as the marketed eye drops were placed in separate petri plate bores under aseptic conditions. A positive control (petri plate with microorganism but placed in normal saline) and a negative control (petri plate without microorganism) were also prepared. [21-23]

Evaluation

Appearance, Clarity & pH

All the developed formulations were observed cautiously for colour and presence of suspended particulate matter assuming any. The clarity of solutions was further assessed & found that F7, F8 & F9 formulations were not clear whereas rest were low cloudy appearance. The pH of all ophthalmic formulations was found as shown in Table 3.

Table 3: Appearance, Clarity & pH

Formulation	Appearance	Clarity	pH
F1	Translucent	+	6.9
F2	Translucent	+	7.1
F3	Translucent	+	7.2
F4	Translucent	+	6.8
F5	Translucent	+	6.9
F6	Translucent	+	7.0
F7	Cloudy	-	6.5
F8	Cloudy	-	6.7
F9	Cloudy	-	6.8

Particle Size, Polydispersity Index & Zeta Potential

The PDI of the formulations was varied from 0.137 to 0.512. Particle size of formulation varies from 183.7 nm to 962.1 µm as shown in table 4. The zeta potential of the formulations was varied from 14.2 to 27.5 as shown in Table 4.

Entrapment Efficiency (EE) & Loading Capacity (LC)

The percent Entrapment efficiency of all formulations were found to be in the range of 39.80 to 94.24 % as shown in Table 4. The higher entrapment of Moxifloxacin in nanoparticles could be contributed to greater retentivity of Moxifloxacin in Chitosan-TPP matrix. The percent loading capacity of all formulation were found to be in the range of 3.32 to 7.87 as shown in the Table 4.

Table 4: Evaluation Parameters of Nanoparticles

Formulation	Entrapment Efficiency (%)	Loading Capacity (%)	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	75.02	6.27	257.5	0.290	25.2
F2	84.63	7.07	183.7	0.428	27.5
F3	65.42	5.46	341.9	0.350	26.1
F4	59.01	4.93	378.3	0.512	19.5
F5	52.61	4.39	457.8	0.430	17.6
F6	39.80	3.32	489.2	0.137	21.3
F7	91.03	7.60	750.2	0.380	15.5
F8	94.24	7.87	962.1	0.346	14.2

***In-Vitro* Studies**

The medium used was freshly prepared Phosphate buffer (pH 7.4). At time intervals of 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr & 24 hr respectively, samples were withdrawn and replaced to maintain sink conditions. In-vitro release pattern of different Moxifloxacin loaded Chitosan-TPP nanoparticles is shown in Table 5 and Figure1.

Table 5: *In-vitro* Drug Release of Different Formulations

Time (hr)	% Cumulative Drug Release								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
0.25	8.645	9.768	6.225	5.225	4.875	6.075	12.068	18.388	21.388
0.5	16.233	17.356	13.813	11.813	10.513	11.813	23.606	28.966	32.931
1	23.029	24.152	20.609	17.609	15.309	17.409	30.502	39.737	46.822
2	33.403	36.526	30.983	26.983	23.573	26.193	47.18	59.801	64.5
4	48.862	51.985	44.442	39.442	34.239	37.859	67.665	85.627	87.027
8	65.308	68.431	60.888	54.888	46.278	50.408	87.787	89.96	90.23
12	76.73	79.853	72.31	65.31	56.157	61.457	91.54	-	-
24	89.603	92.726	85.183	77.183	65.983	74.233	-	-	-

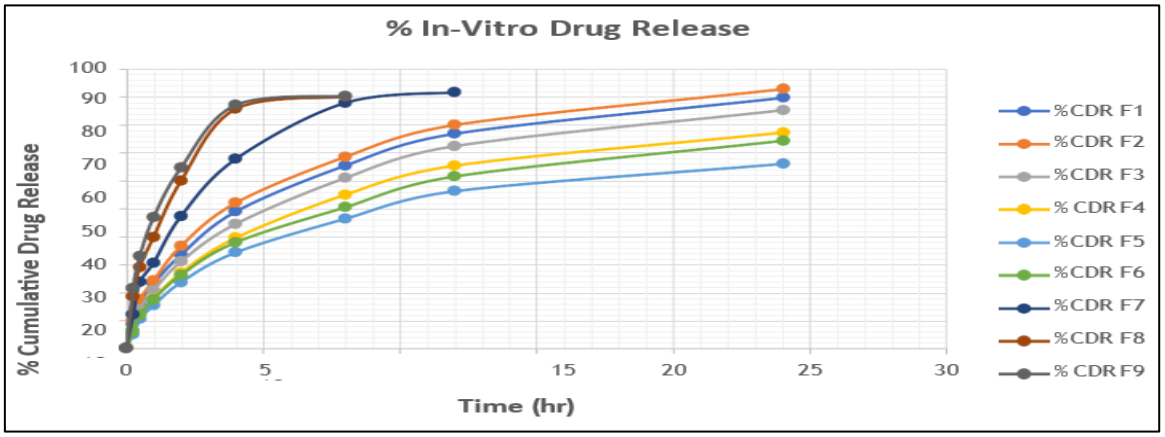

Figure 1: *In-Vitro* Cumulative Drug Release of Different Formulation

Determination of Drug Release Kinetics

The release from nanoparticle formulation after being subjected to different model

dependent kinetics (zero-order, first-order, Higuchi model, Korsmeyer-peppas model & Hixon-Crowell model) was evaluated for their R^2value (Table 6). Hence, formulation shows different release model.

Table 6: Correlation Coefficients (R^2) of Different Nanoparticle Formulation

Formulation	Zero order	First order	Higuchi	Korsmeyer Peppas	Hixon-Crowell
F1	0.8236	0.9766	0.9635	0.9739	0.9772
F2	0.8144	0.9855	0.9561	0.9761	0.9772
F3	0.8295	0.9655	0.9664	0.9795	0.9764
F4	0.8338	0.9441	0.9683	0.9775	0.9764
F5	0.8348	0.9186	0.9686	0.9798	0.9751
F6	0.8525	0.9516	0.9766	0.9868	0.9751
F7	0.9034	0.9985	0.9850	0.9914	1
F8	0.8720	0.9951	0.9694	1	0.9537
F9	0.8465	0.9982	0.9586	1	0.9564

***Ex-vivo* Trans-corneal Permeability**

Results indicated that the inclusive of MOX in the colloidal system considerably increased the penetration rate of the drug across the cornea. MOX loaded Chitosan-TPP nanoparticles showed a significantly higher drug permeation capability. This favourable penetration of MOX across the cornea could be attributed to the agglomeration of nanoparticles in conjunctival sac, thus forming depot from which the drug is slowly delivered to the precorneal area.

Table 7: *Ex-vivo* Trans-corneal Permeation Data of Different Formulation

Time (hr)	Drug Permeation (%)								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
0.5	3.11	6.23	3.33	2.63	2.33	2.1	1.87	1.64	1.41
1	11.29	14.41	11.51	10.81	10.51	10.28	10.05	9.82	9.59
2	19.64	22.76	19.86	19.16	18.86	18.63	18.4	18.17	17.94
3	25.46	31.83	23.53	22.73	21.98	21.75	18.52	18.27	18.19
4	38.15	44.52	36.22	35.42	34.67	34.44	18.79	18.43	18.31

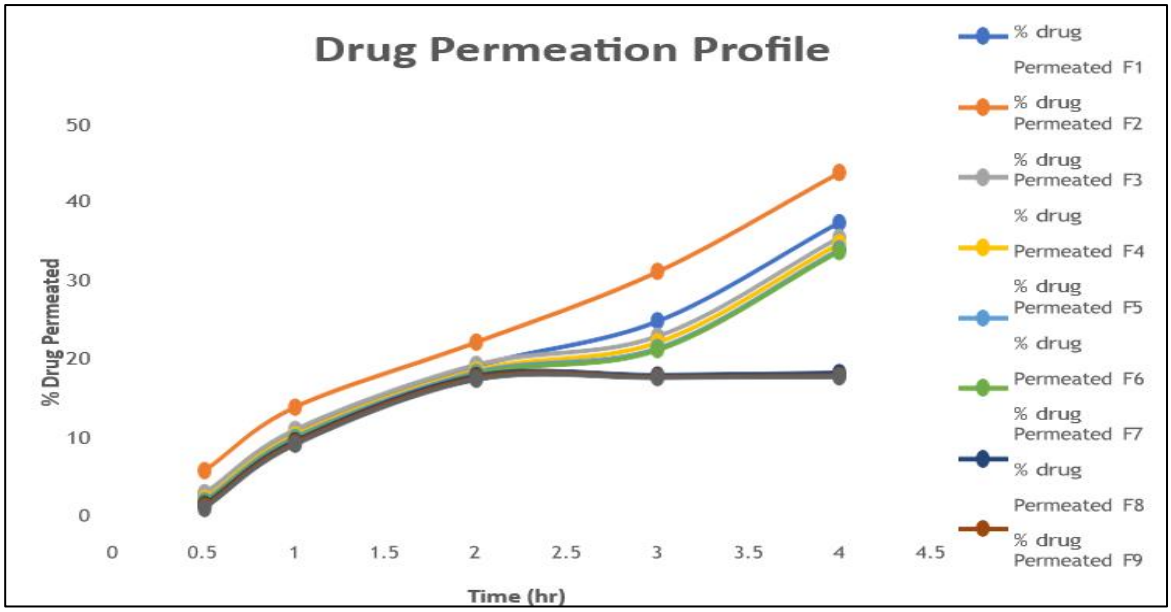

Figure 2: *Ex-vivo* Trans-corneal Permeation Profile of Different Formulation

Optimization of Nanoparticles

The optimized formulation (F2) has 183.7 nm particle sizes which are acceptable for nano-formulation. The optimized formulation has +27.5 mV Zeta potential which stabilize the formulation. The maximum entrapment efficiency was found for F2 (84.63%) & comparatively high loading capacity was found for F2 (7.07%) which was stable & has nano range particle size. Some other formulation has better entrapment efficiency but they exceed the nano range of particle size (F7, F8 & F9) because of that they are not suitable for ophthalmic preparation.

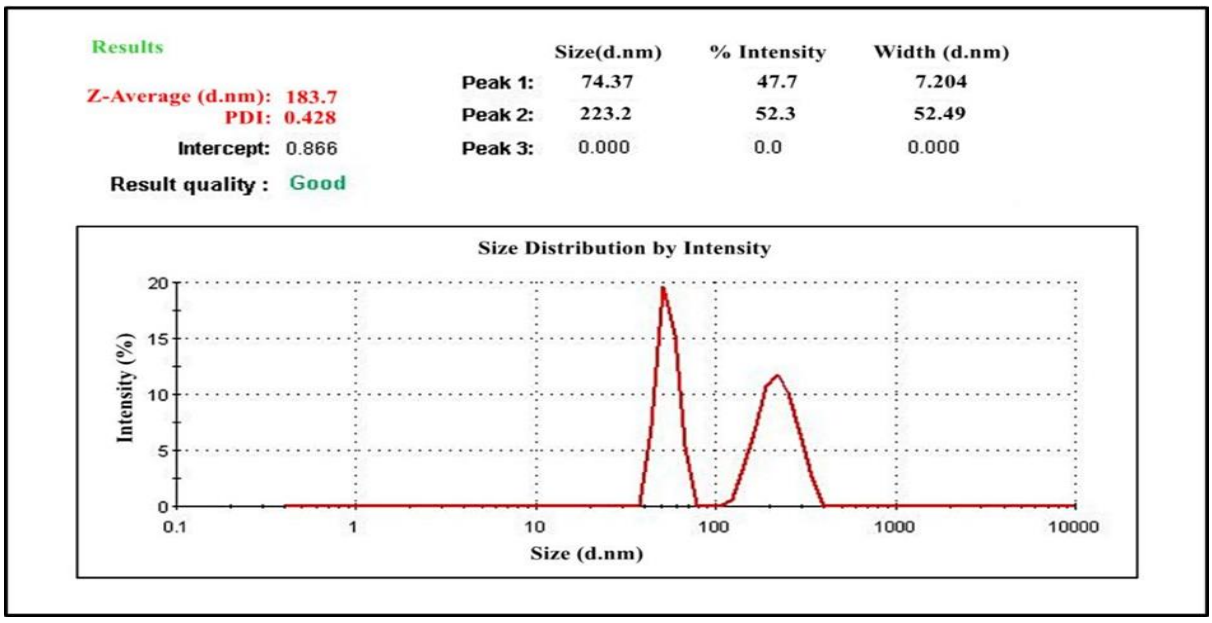

Figure 3: Particle Size of Optimized Formulation (F2)

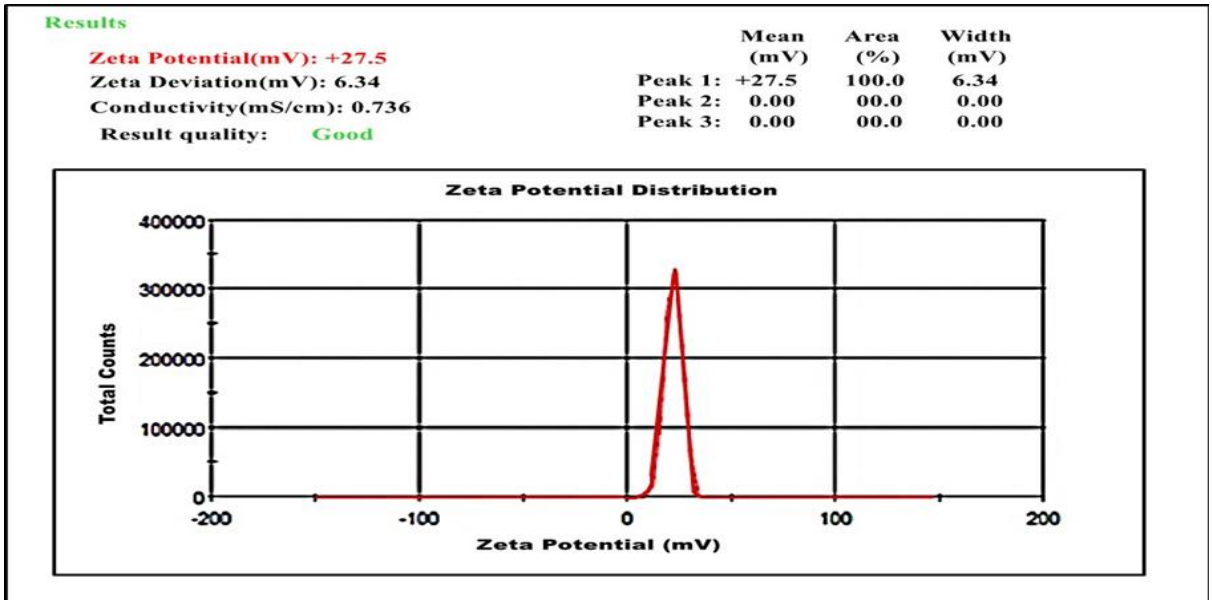

Figure 4: Zeta Potential of Optimized Formulation (F2)

The optimized formulation (F2) follows First order release model which means Drug release rate depends on the concentration. Matrix dissolution-controlled release, Matrix diffusion-controlled release, Solutions & Sustained release formulation follows First Order Release System.

Figure 5: First-order Release Kinetic Model of Optimized Formulation (F2)

Surface Morphology

The drug loaded nanoparticles of formulation F2 was found to be spherical with a smooth surface (Figure 6).

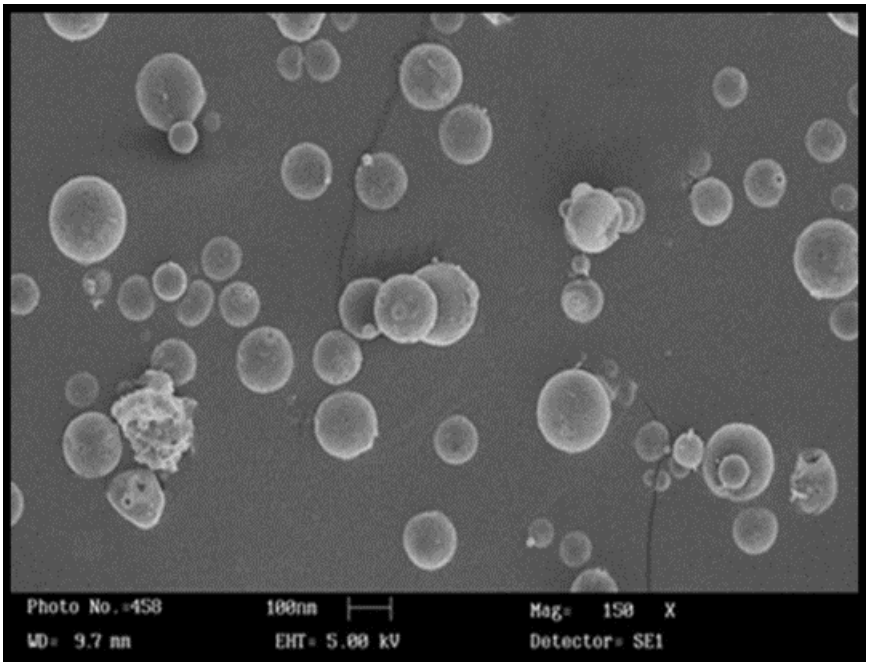

Figure 6: SEM of Optimized Formulation (F2)

Transmission Electron Microscopy (TEM)

Representative TEM image of nanoparticles demonstrates spherical and discrete vesicles of < 300 nm of formulation. The TEM image of optimized nanoparticles is shown in Figure 7.

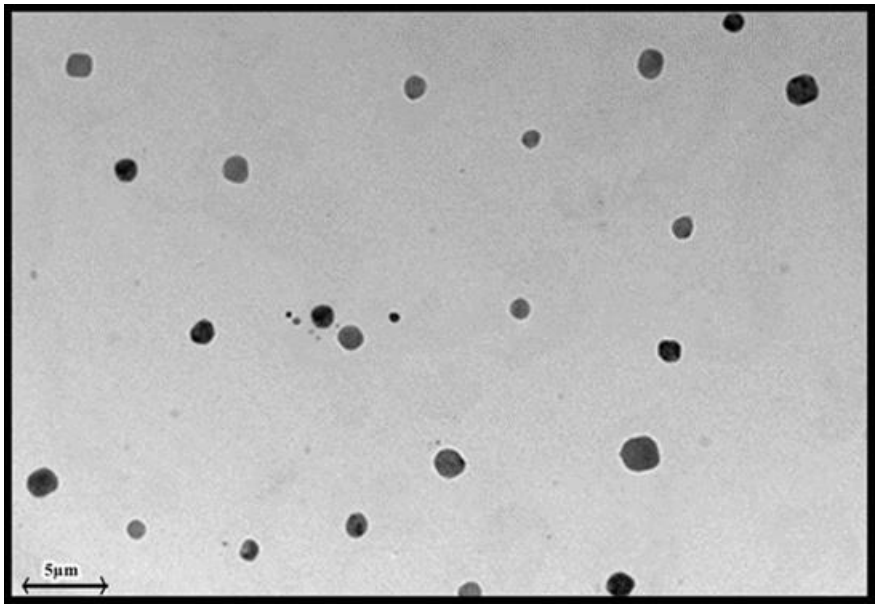

Figure 7: TEM of Optimized Formulation (F2)

Microbiological Study

Optimized formulation (F2) & marketed eye drops of Moxifloxacin HCl were evaluated for antimicrobial activity by cup-plate method. Formulation (F2) gave a clear zone of inhibition comparable with the zone of inhibition given by marketed eye drops. Results showed that formulation has better antimicrobial efficacy compared with the marketed eye drops. Results obtained were compared with the control (without drug) as shown in Figure 8 & 9.

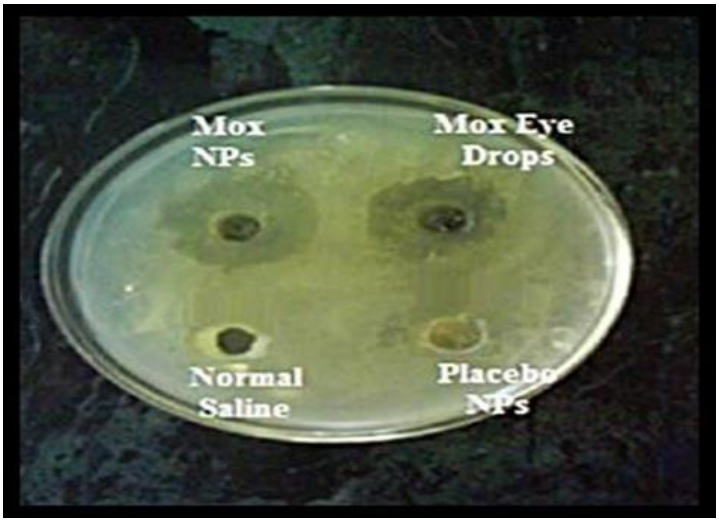

Figure 8: Antimicrobial Activity against *Pseudomonas aeruginosa*

Figure9: Antimicrobial Activity against *Staphylococcus aureus*

Conclusion

Formulations of Moxifloxacin are available in the form of eye drops and eye ointments in the market. When administered as eye drops, many reports were found regarding poor bioavailability because of solution drainage, rapid precorneal elimination and tear turnover. When ointment is applied topically to the cornea, blurred vision is the major problem, which may result in reduced patient compliance. This leads to frequent instillation of concentrated medication to achieve the desired therapeutic effect. Therefore, it is necessary for research scientists to develop an ocular drug delivery system which will overcome and reduce side effects associated with conventional ocular preparations with better bioavailability.

References

1. Sasaki, H., Yamamura, K., Nishida, K., Nakamurat, J., Ichikawa, M., (1996), "Delivery of Drugs to the Eye by Topical Application," Progress Retinal & Eye Res, 15(2), pp. 553-620.
2. Kaur, I. P., and Kanwar, M., (2002), "Ocular Preparation: The Formulation Approach," Drug Development and Industrial Pharmacy, 28(5), pp. 473-493.
3. Cross, J. T., (2001), "Fluoroquinolones," Seminars in Paediatric Infectious Diseases, Elsevier, 12(3), pp. 211-23.
4. A.G. Cuenca, H. Jiang, S.N. Hochwald, M. Delano, W.G. Cance, S.R. Grobmyer, Emerging implications of nanotechnology on cancer diagnostics and therapeutics. Cancer, 107 (2006) 459-66.
5. R.L. Manthe, S.P. Foy, N. Krishnamurthy, B. Sharma, V. Labhasetwar, Tumor ablation and nanotechnology. Mol Pharm, 7 (2010) 1880-98.
6. A.Z. Wang, R.S. Langer, O.C. Farokhzad, Nanoparticle delivery of cancer drugs. Annu Rev Med 2011.
7. Shastri, D. H., Prajapati, S. T., Patel, L. D., (2010), "Thermoreversible Mucoadhesive Ophthalmic In-Situ Hydrogel: Design and Optimization Using a Combination of Polymers," Acta Pharmaceutica, 60, pp. 349-360.
8. El-Laithy, H. M., Nesseem, D. I., Shoukry, M., (2011), "Evaluation of Two In-Situ Gelling Systems for Ocular Delivery of Moxifloxacin: In Vitro and In Vivo Studies," Journal of Chemical & Pharmaceutical Research, 3(2), pp. 66-79.
9. Shastri, D. H., Prajapati, S. T., Parikh, R. K., Patel, L. D., (2009), "Studies on Poloxamer Based Mucoadhesive Ophthalmic In-Situ Hydrogel of Moxifloxacin HCl," International Journal of Pharmaceutical Research, 1(3), pp. 77-86.
10. Kleinmann, G., Apple, D., Chew, J., Mamalis, N., Olson, R. J., (2006), "Hydrophilic Acrylic Intraocular Lens as a Drug Delivery System for the Fourth-Generation Fluoroquinolones," Journal of Cataract & Refractive Surgery, 32(10), pp. 1717-1721.
11. Kleinmann, G., Larson, S., Hunter, B., Stevens, S., Mamalis, N., Olson, R. J., (2007), "Collagen Shields as a Drug Delivery System for the Fourth-Generation Fluoroquinolones," Ophthalmologica: International Journal of ophthalmology, 221(1), pp. 51-56.
12. Islam, S., Rahman Bhuiyan, M. A., Islam, M. N., (2017), "Chitin and Chitosan: Structure, Properties and Applications in Biomedical Engineering," Journal of Polymers and the Environment, 25, pp. 854–866.
13. Smyth, M. S., and Martin, J. H. J., (2000), "X-Ray crystallography", Molecular Pathology, 53 (1), pp. 8–14.
14. Aulton, M., (2007), "Aulton's Pharmaceutics: The Design and Manufacture of Medicines," Elsevier, 3, pp. 336-360, 106-107 and 661-665.
15. Berne, B. J., and Pecora, R., (1976), "Dynamic Light Scattering," The British Polymer Journal, John Wiley & Sons, New York, 9 (2), pp. 376.
16. Choi, H., Jung, J., Ryu, J., Yoon, S., Oh, Y., Kim, C., (1998), "Development of In Situ- Gelling & Mucoadhesive Acetaminophen Liquid Suppository," International Journal of Pharmaceutics, 165(1), pp. 33-44.

17. Charoo, V., Kohli, K., Ali, A., (2003), "Preparation of In Situ-Forming Ophthalmic Gels of Ciprofloxacin Hydrochloride for the Treatment Of Bacterial Conjunctivitis: In Vitro & In Vivo Studies," Journal of Pharmaceutical Sciences, 92(2), pp. 407-413.
18. Srividya, B., Cardoza, R., Amin, P., (2001), "Sustained Ophthalmic Delivery of Ofloxacin from a pH Triggered In-Situ Gelling System," Journal of Control Release, 73(2-3), pp. 205-211.
19. Gonjari, I., Karmarkar, A., Khade, T., Hosmani, A., Navale, R., (2010), "Use of Factorial Design in Formulation & Evaluation of Ophthalmic Gels of Gatifloxacin: Comparison of Different Mucoadhesive Polymers," Drug Discovery and Therapy, 4(6), pp. 423-434.
20. Puglia, C., Bonina, F., Trapani, G., Franco, M., Ricci, M., (2001), "Evaluation of In Vitro Percutaneous Absorption of Lorazepam & Clonazepam from Hydro-Alcoholic Gel Formulations," International Journal of Pharmaceutics, 228(1-2), pp. 79-87.
21. Korsmeyer, R., Gurny, R., Doelker, E., Buri, P., Peppas, N., (1983), "Mechanisms of Solute Release from Porous Hydrophyllic Polymers," International Journal of Pharmaceutics, 15(1), pp. 25-35.
22. Dandagi, P. M., Belekar, A. M., Mastiholimath, V. S., Gadad, A. P., Sontake, V. W., Salian,
23. P. S., (2013), "An Improvement of the Efficacy of Moxifloxacin HCl for the Treatment of Bacterial Keratitis by the Formulation of Ocular Mucoadhesive Microspheres", Sci Pharm, 81, pp. 259–280.