IJPSR (2020), Volume 11, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 November 2019; received in revised form, 07 September 2020; accepted, 20 October 2020; published 01 November 2020

DEVELOPMENT AND EVALUATION OF MOXIFLOXACIN HYDROCHLORIDE LOADED POLY LACTIC-CO-GLYCOLIC ACID NANOPARTICLES FOR OCULAR DRUG DELIVERY

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Keywords:

Moxifloxacin hydrochloride, Poly lactic-co-glycolic acid, Nano-particle, ocular drug delivery

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ABSTRACT: In general, ocular efficacy is strongly related to ocular drug bioavailability, which may be enhanced by prolonging precorneal drug residence time and increasing corneal drug penetration. Hence, the present study involves the development, characterization and evaluation of biodegradable moxifloxacin hydrochloride nano-particles intended for ocular use. Nano-particles were prepared by nano-precipitation technique using poly lactic-co-glycolic acid. 32 factorial designs were applied to optimize the drug formulation. The effect of independent variables such as drug-to-polymer ratio and speed of homogenizers on entrapment efficiency and particle size were investigated. Studies such as X-ray diffraction (XRD), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM) were carried out on the selected formula. *In-vitro* release study showed extended drug release. Hen's egg chorioallantoic membrane (HET-CAM) test was used for the evaluation of ocular tolerance, which showed the non-irritant efficacy of the developed formulation. These results express the applicability of encapsulating moxifloxacin hydrochloride biodegradable polymeric nanoparticles for ocular delivery.

INTRODUCTION: Moxifloxacin is an 8-methoxy fluoroquinolone having a half-life nearly 12 h. It has a broad-spectrum antibiotic activity, with efficacy against various Gram-positive and Gram-negative microorganisms through inhibition of DNA gyrase and topoisomerase IV, and is indicated for treating bacterial conjunctivitis [1]. As compared to other fluoroquinolones, moxifloxacin has the highest potency against *Staphylococcus aureus* and *Staphylococcus epidermis*.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(11).5685-93

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(11).5685-93

Ocular drug delivery systems are developed to treat eye locally, whereas past formulations are targeted to reach systemic circulation and these are designed to overcome all the disadvantages of conventional dosage forms such as ophthalmic solutions [2].

The major difficulty with conventional dosage forms is eye irritation (due to drug particle size and shape), which induces lacrimation, *i.e.*, overflow on to lids, tear turn over, and due to pharmacokinetic responses like metabolism, non-specific binding, and different mechanisms like diffusion, dissolution, and erosion the conventional dosage forms are less advantageous [3]. The conventional dosage form, *i.e.*, eye drops are easy to inculcate but suffers from the natural disadvantage that the majority of the medication it contains is immediately diluted in the tear film as soon as the

eye drop solution is instilled into the cul-de-sac and is quickly drained away from the precorneal cavity by steady tear flow, a process that proceeds more intensively in inflamed than in the normal eyes, and lacrimal-nasal drainage [4].

Ocular efficacy is strongly related to ocular drug bioavailability, which may be improved by increasing corneal drug penetration and prolonging precorneal drug residence time. A variety of ocular drug delivery systems such as inserts and collagen shields and colloidal systems such as liposomes, nanoparticles, and nanocapsules have been designed and investigated for enhanced ocular bioavailability.

The use of nanotechnology-based drug delivery systems such as micro-emulsions, nano-suspension, nano-particles, solid lipid nano-particles, niosomes, dendrimers, and liposomes has led to the solution of various solubility-related problems of poorly soluble drugs [5]. Polymeric nano-particle formulation is one of the strategies presently used to improve drug absorption across biological membranes.

Taking into consideration the fact that there is a short residence of the dosage form in the ocular cavity, it was proposed that the use of mucoadhesive polymers that increase the concentration and residence time of the associated drug [6]. Based on literature data, the three most commonly used polymers in ophthalmic drug formulations are poly (alkyl cyanoacrylates), polycaprolactone, and Poly D, L-lactide.

The literature facts also reveal that in the case of ophthalmic drug delivery, suitable particle size and a narrow size range, ensuring low irritation, enough bioavailability, and compatibility with ocular tissues, should be required for every suspended drug. Among the wide variety of mucoadhesive polymers reported in the literature, the Poly D, L-lactide has been selected as a polymer of choice because of its distinctive properties, including acceptable biodegradability, biocompatibility as well as the ability to increase membrane permeability [7].

So, the present work was designed towards the development and characterization of biodegradable nanoparticles containing moxifloxacin hydrochloride to improve precorneal residence time and ocular bioavailability.

MATERIALS AND METHODS: Moxifloxacin hydrochloride was received from Cipla Ltd, Mumbai, India, as a gift sample. Polyvinyl alcohol (PVA) M. Wt. 22000 Dialysis tubing cellulose membrane which is having molecular weight cut-off 12000-14000 g/mole was purchased from Sigma Aldrich Pvt Ltd. Mumbai, India. Poly lactic-co-glycolic acid ester having a viscosity of 0.16-0.24dL/g was purchased from Sigma Aldrich Pvt. Ltd. Mumbai. All other reagents used were of analytical grade.

Preparation of Drug Loaded Nanoparticles using the Nano-precipitation Technique: The nano-precipitation technique was used for the preparation of moxifloxacin hydrochloride nanoparticles. Organic solution of biodegradable polymer (Poly lactic-co-glycolic acid) and the exact amount of Moxifloxacin Hydrochloride 50 mg in 10 mL of acetone were prepared. The organic phase was added dropwise into 20 mL of an aqueous solution containing PVA (1%) and stirred magnetically. After 30 min of stirring the volume of nanoparticles dispersion was concentrated to 10 mL under reduced pressure using a Rota evaporator with a vacuum (KNF, vaccum pumps & system). The aggregates were removed by filtration through a 0.45 µm syringe filter. Separation of the non-encapsulated drug was performed by ultra-centrifugation (Beckman Coulter) at 50,000 rpm at 4 ºC for 30 min. The supernatant was discarded, and separated nanoparticles were washed twice with distilled water to remove excess surfactant. The washed particles were resuspended in 5 mL of a water solution containing 5% (w/v) mannitol as cryoprotectant and freeze-dried for 48 h. The whole experiment was carried out in an aseptic area. The nanoparticles were kept at 2 to 8 ºC for further investigation.

Design of the Experiment: 3^2 full factorial design was used to study the effect of different parameters on the physicochemical properties of the prepared nanoparticles. The concentration of polymer and different speeds were selected as the independent variables. The particle sizes of the colloid system and the encapsulation efficiency of the drug were selected as the dependent variables.

TABLE 1: EXPERIMENTAL PLAN OF THE 32 FACTORIAL DESIGNS

Factor	Level		
	++	+	-
Polymer Concentration (mg)	150	100	50
Speed of Homogenizer	1200	1000	800

TABLE 2: COMPOSITION OF DRUG /POLYMER CONCENTRATION AND SPEED OF DIFFERENT FORMULATION OF OPHTHALMIC POLYMERIC NANOPARTICLES

Formula Number	Drug: Polymer Concentration	Speed
F1	1:1	800
F2	1:2	800
F3	1:3	800
F1	1:1	1000
F5	1:2	1000
F6	1:3	1000
F7	1:1	1200
F8	1:2	1200
F9	1:3	1200

Characterization of the Nanoparticles:

Determination of the Particle Size: Photon correlation spectroscopy with Zeta-particle size, Model Nano ZS was used for the determination of Size distribution, average particle size, and PDI. The separated nanoparticles were subjected to measurement followed by dilution with distilled water. The particle size and PDI measurements were carried out at a scattering angle of 900 and at a temperature 25 °C. All experiments were done in triplicate [8].

Encapsulation Efficiency (EE %) Measurements: For the determination of the encapsulation efficiency of moxifloxacin hydrochloride in polymeric nanoparticles, extraction and quantification of the encapsulated Moxi-floxacin Hydro-chloride was performed. The portion of the encapsulated moxifloxacin hydrochloride was obtained by ultra-centrifugation 1 ml of the nanoparticle suspension at 18000 rpm for 1hour using a cooling centrifuge at 4 °C. The supernatant was removed, and the formed pellets were re-suspended in phosphate buffer saline pH 7.4 to ensure the complete removal of all free moxifloxacin hydrochloride. The supernatant (free moxifloxacin hydrochloride) was collected and measured using HPLC. A reverse phase C-18 column was equilibrated with the mobile phase Ammonium formate: Acetonitrile (70:30) and pH adjusted to 4.0 with formic acid.

The mobile phase flow rate was maintained at 1ml/min, and eluents were monitored at 295 nm for moxifloxacin. The sample was injected using a 20 µl fixed loop. The mobile phase was prepared by mixing 700 ml of 20 mM ammonium formate solution with 300 ml of HPLC grade acetonitrile to get the proportion of 70:30 v/v, and finally, the pH was adjusted to 4.0 with formic acid. The mobile phase was sonicated for 10 min and filtered through a 0.45 µ membrane filter. The entrapped moxifloxacin hydrochloride concentration was expressed as percentage entrapment efficiency, which can be defined as the percent fraction of the total input drug encapsulated in the polymeric nanoparticles [9].

EE of the drug = (amount of encapsulated drug) / (total amount of the drug) × 100 ……Equation no.1

Redispersibility of Nanoparticles: To obtain a dry powder for further investigation, the selected formulation was freeze-dried. In addition to that, it was taken to study the effect of cryoprotectant on freeze-drying and dispersibility of the prepared nanosuspension. Mannitol at a concentration of 5 times the total solid contents in the formulation used as a cryoprotectant. Two samples of nano-suspension each were placed in a flask; the amount of mannitol required added to one and shaken to dissolve, the second sample left without cryoprotectants. These flasks were frozen in a deep freezer at -20 °C for 12 h for primary freezing. Then the container was attached to the vacuum adapter of the lyophilizer. The solvent sublimed under a pressure of 80 mm Hg for 48-72 h [10].

Differential Scanning Calorimetry: Differential Scanning Calorimeter (DSC DA 60 Shimadzu, Japan) equipped with a liquid nitrogen subambient accessory was used for differential scanning calorimetric measurements. The DSC was performed for the pure moxifloxacin, the poly lactic-co-glycolic acid, and the drug-loaded nano-particle formulation. Sample 2 mg were loaded in a flat-bottomed aluminium pan and subjected to a heating cycle from 40 to 400 ºC with a heating rate of 10º C/min. A stream of nitrogen gas was used to control the heating and cooling rate. The temperature and energy scales of the instrument were calibrated using purified indium as the reference material [11].

X-ray Diffraction: To detect the crystallinity of the drug in nanoparticle formulation X-ray diffraction analysis was employed, which was conducted using a Philips PW 3710 X-ray diffractometer (XRD) with a copper target and nickel filter. Powders were mounted on aluminium stages with glass bottoms and smoothed to a level surface.

The XRD pattern of each sample was measured from 10 to 50 degrees 2-theta using a step increment of 0.1 2-theta degrees and a dwell time of 1 sec at each step [12].

Scanning Electron Microscopy: To characterize the surface morphology of nanoparticles the scanning electron microscopy (JEOL Model JSM - 6390LV) was used. The nanoparticles were mounted directly on the SEM stub, using double-sided, sticking tape and coated with platinum and scanned in a high vacuum chamber with a focused electron beam. Secondary electrons emitted from the samples were detected and the image formed [13].

Swelling Index: The accurately weighed nano-particles were placed in a glass vial containing pH 7.4 phosphate buffer 10 mL at 37 ± 0.5 °C in incubator and was stirred occasionally. The nano-particles were periodically removed by blot using filter paper and the change in weight of particulates was measured till equilibration. The weight was recorded after a period of 3 h in triplicate, and the swelling ratio (SR) was calculated using formula (Eq. 2) [14].

$$\text{Swelling index (\%)} = W_1\text{-}W_2 \times 100 / W_1 \quad \text{..... Equation no. 2}$$

Where, W_1 = Weight of nano-particles after swelling, W_2 = Initial weight of nano-particles

Sterility Testing: To ensure the sterility of finished product, Sterility studies were carried out. Ever since it is administered by the parenteral route, direct inoculation method was chosen to carry out sterility testing. In this method, the specified quantity of sample under test was drawn aseptically from the containers and transferred to fluid thioglycollate medium (20 mL) and Soybean-Casein digest medium (20 mL), separately. A mixture of nano-particles with the medium was incubated for not less than 14 days at 30 °C–35 °C in case of fluid thioglycollate medium and 20 °C–25 °C in case of Soybean-Casein digest medium. The growth of any microorganisms in the medium was observed [15].

***In-vitro* Release Studies:** A membrane diffusion technique was used to carry out the release study of nanoparticle formulation for the release of moxifloxacin hydrochloride from polymeric nanoparticles. *In-vitro* diffusion cell was made using dialysis membrane as a semi-permeable membrane. To retain the nanoparticles as well as to enable the free drug to diffuse freely into the release media a dialysis membrane of 12,000-14,000 Molecular weight cut-off was used.

Formulated nanoparticles equivalent to 1 mg of the drug were dispersed in 1 mL of isotonic phosphate-buffered saline at pH 7.4. The nanoparticle dispersions were packed in a dialysis membrane secured with two clamps at each end. To maintain sink condition, the dialysis bag was immersed in tightly-capped glass vials (7 × 2.8 cm) containing 10 mL of 0.5% (w/v) of sodium lauryl sulphate solution in distilled water. The release test was performed by placing the glass vials in a thermostatically-controlled shaking water bath adjusted to 37 ± 0.5 °C with a constant shaking rate of 100 rpm. At predetermined time points, the whole release medium was withdrawn and replaced with a fresh release medium. The concentration of the drug released was measured by HPLC. All experiments were carried out in triplicate [9].

Chorioallantoic Membrane (HET-CAM) Test and Irritation Score Calculation: For Draize eye irritation test, a chorioallantoic membrane (CAM) testing as a mucous-membrane irritation test was performed. Commercially available fertilized white chicken eggs without micoplasms were used for the test. For CAM testing, the hen's eggs were put in incubator trays with the large ends up; the trays were placed in the incubator, which automatically rotates and was maintained at an optimum temperature of 37.5 ± 0.5 °C. The eggs were candled on day 5 of incubation, and every day thereafter; nonviable embryos were removed. On day 10 of incubation, the eggshell was scratched around the air cell by a dentist's rotary saw and then pared off. After careful removal of the inner egg membranes, the vascular CAM was exposed. The test sample in a volume of 0.2 ml was applied on

the CAM surface. A series of four eggs was used; two eggs, treated with vehicle only, serve as controls. After the application of the test substance, the CAM, the blood vessels, including the capillary system and the albumen, were examined and scored for irritant effects (hyperemia, hemorrhages, coagulation) at 0.5, 2, and 5 min after treatment.

The time-dependent numerical scores for hyperemia, hemorrhages, and coagulation **Table 3** were summed to give a single numerical value indicating the irritation potential of the test substance on a scale with a maximum value of 21. The mean value of four tests makes possible an assessment by a classification scheme analogous to the Draize categories **Table 4** [16].

TABLE 3: SCORING SCHEME FOR IRRITATION TESTING WITH THE HEN'S EGG CHORIOALLANTOIC MEMBRANE

Effect	Score (Time in Second)		
	0.5	2	5
Hyperemia	5	3	1
Hemorrhage	7	5	3
Coagulation	9	7	5

TABLE 4: CLASSIFICATION OF CUMULATIVE SCORES IN THE CHORIOALLANTOIC MEMBRANE TEST

Cumulative irritation	Score assessment
0-0.9	Practically none
1-4.9	Slight
5-8.9	Moderate
9-21	Strong

Stability Studies: Stability studies were carried out on the selected formulation at 30 ± 2 °C in a stability chamber (Thermo lab) for 6 months. The optimized formulation is stored in a sealed in a glass bottle. After 6 months, drug content, particle size, and redispersibility studies were carried out [17].

Results and Discussion:

Experimental Design: Based on the experimental and above studies, two factors (Concentration of polymer and speed of the homogenizer) were determined crucial factors for particle size and entrapment efficiency of the prepared polymeric nanoparticles. The concentration of polymer was found to have a significant effect on entrapment efficiency as the optimum concentration of polymer. The speed of the homogenizer is also an effect on optimization formulation [18].

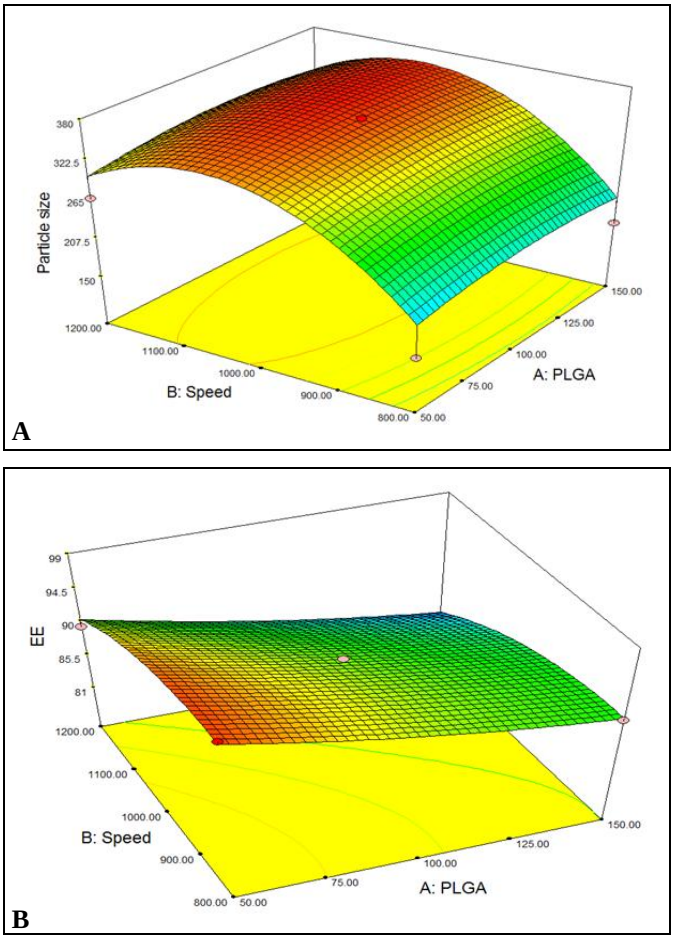

FIG. 1: SURFACE PLOTS SHOWING THE EFFECT OF (A) ENCAPSULATION EFFICIENCY (B) PARTICLE SIZE

Preparation of Drug Loaded Nanoparticles: Moxifloxacin hydrochloride loaded poly lactic-coglycolic acid Polymeric nanoparticles were successfully prepared by using nanoprecipitation technique as it is rapid and easy to perform. Nano-particle formation is one step and instantaneous procedure. When the polymer solution is added to the non-solvent, rapid desolation occurs, followed by nano-precipitation. As soon as the polymer-containing solvent has diffused into the dispersing medium, the polymer precipitates, involving immediate drug entrapment. Moreover, this method always produces a carrier size in the nanometer range and uses ingredients with low toxic potential which is suitable for the ocular route.

Characterization of Nano-particles:

Determination of Particle Size: The particle size is an important factor in the development of an ocular drug delivery system when considering irritation and comfort.

The mean particle size of prepared nanoparticle formulae is shown in the table. The particle size varies from 157.4 to 370.2 nm. The effect of different formulation variables, namely polymer concentration, and speed, had a significant effect on particle size. Concerning the effect of polymer concentration **Table, 5** show that the particle sizes obtained by using lower polymer concentration were significantly smaller than those obtained by using higher polymer concentration.

TABLE 5: PARTICLE SIZE AND ENCAPSULATION EFFICIENCY WITH DIFFERENT FORMULATION

Formulations	Particle size (nm)	Encapsulation efficiency (%)
F1	157.4	97.2
F2	169.7	91.8
F3	177.3	89.6
F1	364.1	98.1
F5	357.8	91.3
F6	370.2	88.7
F7	267.9	89.6
F8	280.7	84.3
F9	294.5	81.3

It showed that the particle size also increases with an increase in polymer concentration. Different speed also affects the particle size, as shown in **Table 5**. Particle sizes obtained by using low speed were significantly larger than the particle sizes obtained by using high speed.

But it is to be noted that if speed is further increased, it did not decrease the particle size to a significant extent. So the optimum speed which gave the smaller particle size was the speed used for F1. Particle size distribution represented by poly-dispersity index (PDI) was measured for all nano-particles formulations. The mean PDI values range from 0.555 to 1.0. The narrow distribution represented by the small PDI values denotes particle size uniformity in the nano-particle formulae.

Encapsulation Efficiency (EE): Nano-particle encapsulation efficiency % ranged between 81.3 to 98.1%. All tested variables have a significant effect on EE%. It was observed that the increase in polymer content resulted in a decrease in EE% of the nano-particles formulae. This result may be attributed to the increasing viscosity of the organic phase upon increasing polymer content.

Redispersibility Test: It was found that when using mannitol as cryoprotectants the dispersibility was improved, and products were spontaneously dispersed into primary nanosuspension within 1-3 min in both media (0.1 N HCl and phosphate buffer pH 6.8). It is recommended that mannitol in the products would improve the wetting of the hydrophobic drug and accelerate the penetration of water into the products. On the other hand, the products without cryoprotectants could not be dispersed well and transformed into the original nano-suspension within 15 min as expected from their agglomerated structure.

DSC: Each sample was analyzed by differential scanning calorimetry (DSC) to verify the existence in the physical interaction between moxifloxacin hydrochloride and excipients. DSC thermogram of Moxifloxacin hydrochloride, poly lacticco-glycolic acid, and lyophilized drug-loaded nanoparticles is shown in **Fig 2**. The characteristic endothermic peaks at 254 ºC of the drug disappeared in the drug-loaded nanoparticles thermogram. It could therefore be concluded that moxifloxacin hydrochloride was entrapped in an amorphous or molecular dispersion state in the polymer matrix.

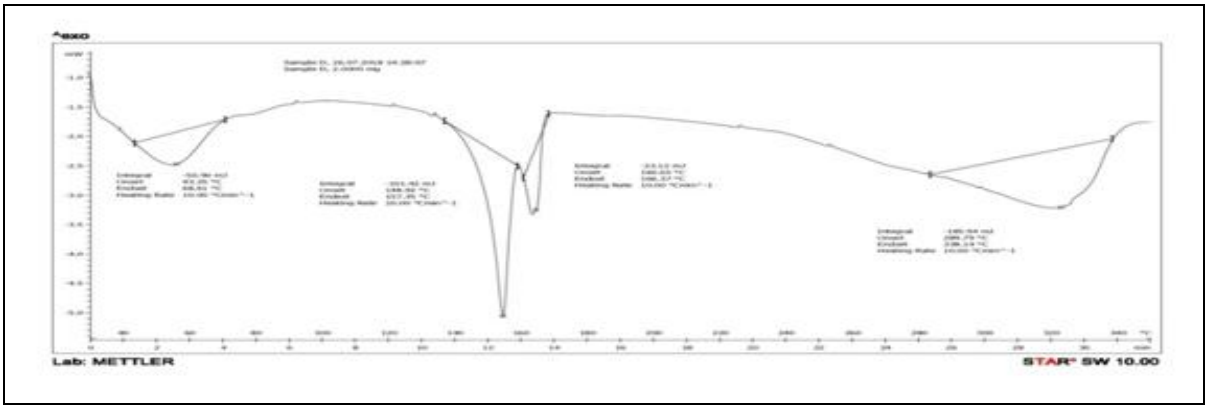

FIG. 2: DSC THERMOGRAM OF THE OPTIMIZED NANOPARTICLES FORMULATION (F1)

XRD: Powder x-ray diffraction was used to determine the crystallinity of the moxifloxacin hydrochloride in the nanoparticles. The powder x ray diffraction patterns of optimized formulation (F1) are shown in **Fig. 3**. It indicates that the drug was present in the nanoparticles in an amorphous state, confirming DSC results.

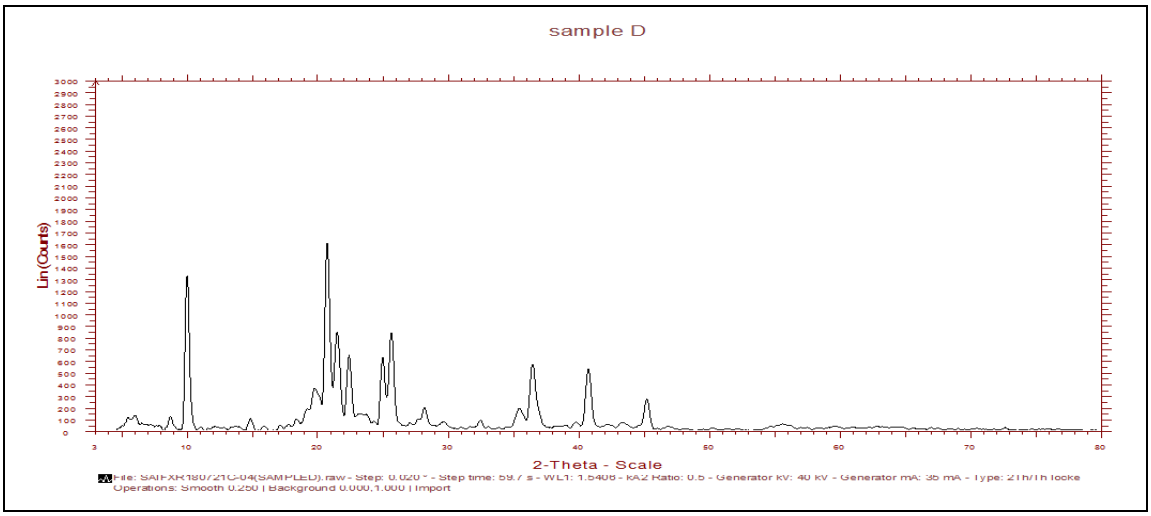

FIG. 3: XRD OF THE OPTIMIZED NANOPARTICLES FORMULATION (F1)

Scanning Electron Microscopy (SEM): The morphology of drug-loaded nanoparticles (F1) was accessed using SEM and is shown in **Fig. 4**. This figure indicates that the nanoparticles were cylindrical in shape, and their size was in the nanometer range with a smooth surface essential for ocular drug delivery.

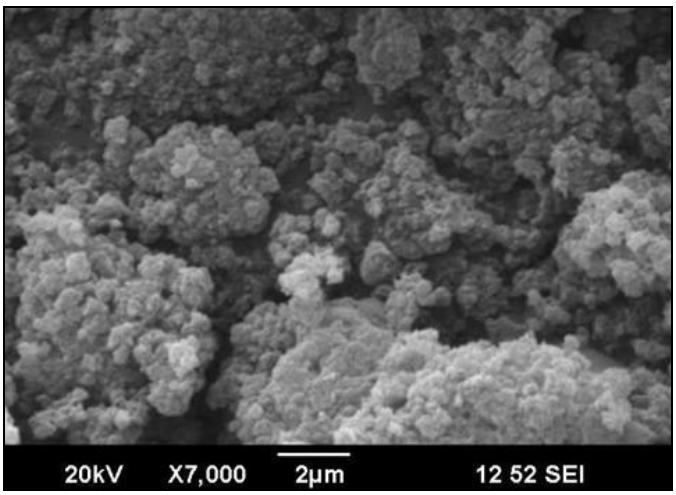

FIG. 4: SEM OF THE OPTIMIZED NANOPARTICLES FORMULATION (F1)

Swelling Index: Exploration increase in swelling index from 55% to 85% as polymer concentration is increase from 50 mg to 150 mg, indicates that being poly lacticcoglycolic acid as hydrophilic polymer uptake excessive amount of water responsible for swelling for polymeric nano-particles.

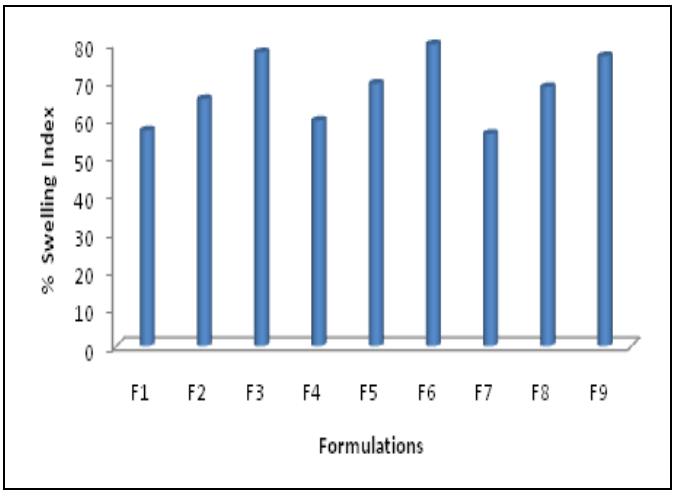

FIG. 5: PERCENTAGE OF SWELLING INDEX OF NANOPARTICLES FORMULATION

Sterility Testing: It was found there was no evidence of microbial growth when formulations were incubated for not less than 14 days at 30 °C to 35 °C in case of fluid thioglycolate medium and at 20 °C to 25 °C in case of Soybean-Casein digest medium demonstrating that formulation passes the test for sterility.

***In-vitro* Drug Release from Nanoparticles:** The drug formulae prepared with nanoprecipitation technique were subjected to an *in-vitro* release study. The amount of Moxifloxacin Hydrochloride released from nanoparticles was evaluated using a dialysis technique. The release profiles of moxifloxacin hydrochloride are shown in **Fig. 6**.

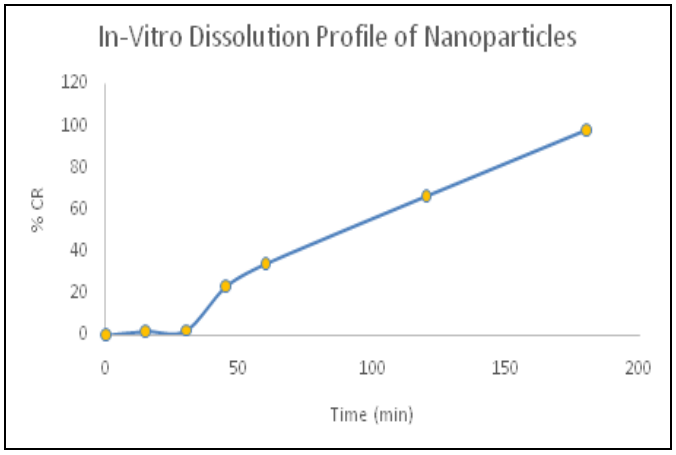

FIG. 6: *IN-VITRO* RELEASE STUDY OF THE MOXIFLOXACIN HYDROCHLORIDE NANO-PARTICLES FORMULATION

CAM Test: It was found that the formulation is non-irritant as score value for Hyperemia, Hemorrhage, and coagulation is zero, as compared to phosphate buffer solution pH 7.4 should coagulation after five minutes (Score value 1.4) which is slightly irritant.

FIG. 7: CAM TEST OF THE OPTIMIZED NANO-PARTICLES FORMULATION (F1)

Stability Studies: Stability studies were carried out on optimized formulation at 30 ± 2 °C in stability chamber (Thermolab) for 6 month. The optimized formulation stored in sealed in aluminium foil. After 6 months, drug content, particle size and redispersibility studies were carried out.

TABLE 6: STABILITY OF MOXIFLOXACIN HYDRO-CHLORIDE NANOPARTICLES DURING STORAGE (F1)

Parameter	0 Day	1 Month	3 Month	6 Month
Drug content	97.2	96.4	95.9	95.1
Particle size	157.4	160.1	161.3	162.4

CONCLUSION: Moxifloxacin hydrochloride was successfully prepared within biodegradable nanoparticle using nanoprecipitation technique. The optimum formulation was obtained by using 3^2 factorial designs. The drug-polymer ratio and speed had a noteworthy effect on the particle size and encapsulation efficiency of the nanoparticle. The formulated moxifloxacin nanoparticles was found to be a suitable and potential natural carrier in terms of their particle size, drug loading capacity, redispersibility, *in-vitro* release characteristics, sterility and better ocular tolerability. The stability study of moxifloxacin from nanoparticles has shown suitable results.

ACKNOWLEDGEMENT: I would like to thanks DCS's A. R. A. College of Pharmacy, Nagaon, Dhule, for the support.

CONFLICTS OF INTEREST: There is no Conflict of interest

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How to cite this article:
Harshal MD, Rajendra WD, Dheeraj BT, Hitendra MS and Bhushan MJ: Development and evaluation of moxifloxacin hydrochloride loaded poly lactic-co-glycolic acid nanoparticles for ocular drug delivery. Int J Pharm Sci & Res 2020; 11(11): 5685-93. doi: 10.13040/IJPSR.0975-8232.11(11).5685-93.