Moxifloxacin Loaded Polymeric Nanoparticles for Sustained Ocular Drug Delivery

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ABSTRACT
Efficient drug delivery to the ocular region is a challenging goal. Only a very small amount (about 1-3%) of the dosage actually penetrates through the cornea and reaches intraocular tissues. To overcome these problems of conventional dosage forms, novel drug delivery systems like nanoparticles were designed. Moxifloxacin-loaded poly (lactic-co-glycolic acid) nanosuspension was prepared with the aim of providing sustained effect for ocular delivery for 24 hours. Nanosuspensions were prepared by nanoprecipitation method using poly(lactic-co-glycolic acid) and evaluated for particle size, surface morphology, zeta potential, drug entrapment efficiency, in vitro release and ex vivo transcorneal permeability, and were compared with marketed products. Microbiological efficacy was tested against Staphylococcus aureus and Pseudomonas aeroginosa using cup plate method. Spherical uniform particles (202.5 nm) with a polydispersity index of 0.226 and negative zeta potential (–25.45 mV) were obtained for MF4 (drug to polymer ratio 1:0.4). Drug entrapment efficiency for MF4 was found to be 83.1%. The cumulative percent drug release for formulation MF4 after 24 hours was 86.1%, showing a sustained effect in controlling the bacterial conjunctivitis thereby avoiding frequent administration of dosage. MFX-loaded PLGA nanoparticles (MF4) showed a significantly higher drug permeation capability compared to the commercial marketed eye drops in ex vivo transcorneal permeation studies and also showed better antimicrobial efficacy compared to the marketed formulation. The results indicate that Moxifloxacin-loaded PLGA nanosuspension could be utilized as a potential drug delivery system for sustained release in ophthalmic application.

KEYWORDS: Moxifloxacin; PLGA nanoparticles; nanoprecipitation method; ex vivo transcorneal permeation.

Introduction
Acute bacterial conjunctivitis is the most prevalent infectious condition, commonly caused by bacterial species like Streptococcus pneumoniae, Haemophilus influenza and Staphylococcus aureus. Newer-generation fluoroquinolones play a major role in the treatment of bacterial conjunctivitis because these agents act quickly against a broad spectrum of pathogens, including most of the bacteria that could be causative agents for bacterial conjunctivitis (Finkel et al., 2009).

With eyes being the most readily accessible organ in the body, achieving good ocular bioavailability is still a challenging task. The bioavailability of ocular drugs in conventional systems that are aqueous solutions is usually low because of quick elimination from the eyes due to reflex blinking and tear drainage. The corneal barrier also plays a significant role in low ocular bioavailability. Nanoparticles come out to be the most promising application in ocular drug delivery (Mandal et al., 2009). Treatment with nanoparticle systems increases bioavailability, reduces administration frequency and promotes drug targeting (Vega et al., 2008).

The most commonly used polymers for ocular nanoparticles are poly(alkyl cyanoacrylate), polycaprolactone, and poly(lactic acid)/poly(lactic-co-glycolic acid) (PLGA). These polymers are biodegradable and undergo hydrolysis in tears (Ding, 1998). Among them PLGA is the most suitable candidate for nanoparticle formation because of its ease of formulation and approval for use in drug delivery application by the Food and Drug Administration (FDA; Jain, 2000). These polymers have a long history of safe human use as a raw material for nanoparticle production of enzyme sensitive drugs (Reis et al., 2008). Poorly water soluble drugs are difficult to develop as a conventional ocular drug delivery system (Patrawale VB et al., 2004). Nanotechnology can be used to formulate such poorly water soluble drugs as a nanosuspension and offers the opportunity to address many of the deficiencies associated with such class of drugs (Kassem MA et al., 2007).

Moxifloxacin, a fourth generation fluoroquinolone antibiotic shows good activity against anaerobic as well as gram-positive organisms (Darlene Miller, 2008). Moxifloxacin HCl is available in the form of drops (0.5% w/v) for ophthalmic use and its FDA-approved dosing

ABBREVIATIONS: Moxifloxacin (MFX); poly-(lactic-co-glycolic acid) (PLGA).
regimen for the treatment of acute bacterial conjunctivitis is 1 drop t.i.d. for 7 days. Moxifloxacin, being a hydrophobic drug, is therefore a suitable candidate for the sustained ocular delivery in order to reduce the frequency of dosing.

The aim of this study was to formulate moxifloxacin-PLGA nanoparticles for sustained ocular delivery and evaluation for parameters like in vitro release, ex vivo transcorneal permeation, and antimicrobial activity.

Material and Methods

Drugs and Chemicals

Moxifloxacin (MFX) was received as a kind gift sample from Orex Pharma Pvt.Ltd. Mumbai. Poly (D, L-lactide-co-glycolide) (75:25) (PLGA) was a gift sample from Purac Biomaterial, Germany. Tween 80 and sodium chloride were purchased from Ranbaxy Fine Chem. Pvt., Ltd., Mumbai. All other chemicals were of analytical grade and used without any further purification.

Compatibility Studies

Compatibility of the drug Moxifloxacin (MFX) and Poly (D, L-lactide-co-glycolide) (75:25) (PLGA), used to formulate nanoparticles was established by IR spectroscopy method. FT-IR spectral measurement for pure MFX drug, polymer PLGA, and physical mixtures of MFX and PLGA were taken at ambient temperature. All the spectra acquired were scanned between 400 and 4000 cm$^{-1}$ at a resolution of 4 cm$^{-1}$.

Preparation of Moxifloxacin Nanoparticles

Moxifloxacin nanoparticles were prepared with a slight modification of the previously reported nanoprecipitation technique (Govender et al., 1999). Typically, different ratios of drug and polymer (keeping drug constant and varying polymer concentrations) were dissolved in acetone (5 ml) at room temperature (Table 2). The solution was mixed and then slowly dropped with a constant speed (0.5 ml/min) into water (20 ml) containing 0.02 w/v of Tween 80 with continuous magnetic stirring at 1800 rpm. Acetone and some water were evaporated, and the final volume of the resulted aqueous nanosuspension was collected. The nanosuspension was then centrifuged at 18,000 rpm, 20°C for 1 hour (Remi, Mumbai, India). The supernatant was discarded and the remaining nanoparticles were collected and washed (three times) with distilled water using centrifugation and finally lyophilized by means of Christ Alpha 1-4 lyophilizator (Christ, Germany) using 1% w/v mannitol as lyoprotectant.

Evaluation of Nanoparticles

**Percentage entrapment efficiency**

To determine the entrapment of MFX in nanoparticles, 1 ml of freshly prepared nanosuspension was taken and diluted with 100 ml STF (pH-7.4). Aliquots of 10 ml were taken and diluted to 100 ml, further subjected to cold centrifuge at ~ 4°C and 15,000 rpm using Sigma 3 K 30 centrifuge for 30 minutes. From the supernatant, a one milliliter solution was taken and diluted appropriately. The resulting solutions were analyzed for MFX content using a double beam UV spectrophotometer and % entrapment efficiency (% EE) was calculated using following equation (Dandagi P et al., 2009).

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

**Percentage yield**

The lyophilized nanoparticles from each formulation were weighed and the respective percentage yield was calculated using the following formula. (Dandagi P et al., 2009).

\[
\text{Percentage Yield} = \frac{\text{Weight of nanoparticles Obtained}}{\text{Weight of drug, polymer and other excipients used}} \times 100
\]

### Table 1

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Drug to Polymer ratio</th>
<th>Mean Particle size (nm±S.D.)*</th>
<th>Polymdispersity Index(PDI ±S.D.)*</th>
<th>% EE (%±S.D.)*</th>
<th>Zeta potential (mV)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF1</td>
<td>1:0.1</td>
<td>174.33±20.3</td>
<td>0.256±4.03</td>
<td>27.03±1.59</td>
<td>-23.50</td>
<td>45.35</td>
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<tr>
<td>MF2</td>
<td>1:0.2</td>
<td>183.98±44.55</td>
<td>0.375±0.06</td>
<td>42.9±1.05</td>
<td>-27.4</td>
<td>47.18</td>
</tr>
<tr>
<td>MF3</td>
<td>1:0.3</td>
<td>194.25±2.89</td>
<td>0.438±0.005</td>
<td>49.6±1.24</td>
<td>-26.38</td>
<td>50.15</td>
</tr>
<tr>
<td>MF4</td>
<td>1:0.4</td>
<td>202.5±3.86</td>
<td>0.226±6.007</td>
<td>83.1±1.19</td>
<td>-25.45</td>
<td>51.81</td>
</tr>
<tr>
<td>MF5</td>
<td>1:0.5</td>
<td>268.5±14.15</td>
<td>0.512±0.002</td>
<td>60.3±1.79</td>
<td>-24.3</td>
<td>62.13</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± S.D. (n=3)*

%EE - % Entrapment Efficiency

### Table 2

<table>
<thead>
<tr>
<th>Functional Groups</th>
<th>Wave Number (cm$^{-1}$)</th>
<th>Moxifloxacin (MFX)</th>
<th>Poly (D, L-lactide-co-glycolide) (PLGA)</th>
<th>MFX + PLGA</th>
<th>(MFX + PLGA)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-F Stretching</td>
<td>1315.45</td>
<td>--</td>
<td>--</td>
<td>1317.38</td>
<td>--</td>
</tr>
<tr>
<td>CH3</td>
<td>1371.39</td>
<td>1750.07</td>
<td>1371.39</td>
<td>1618.28</td>
<td>1618.28</td>
</tr>
<tr>
<td>C=O Carbonyl Stretching</td>
<td>1620.21</td>
<td>--</td>
<td>--</td>
<td>3495.01</td>
<td>--</td>
</tr>
<tr>
<td>NH Stretch</td>
<td>3500.80</td>
<td>2926</td>
<td>3495.01</td>
<td>3583.74</td>
<td>--</td>
</tr>
<tr>
<td>OH Stretch</td>
<td>3545.16</td>
<td>--</td>
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</tr>
</tbody>
</table>

FT-IR Spectral data of pure moxifloxacin (MFX), poly-(D, L-lactide-co-glycolide) and physical mixture of MFX + PLGA.
Particle size, Particle size Distribution and Zeta Potential

The particle size and size distribution along the volume mean diameter of the nanoparticle was measured by a Dynamic Light Scattering Particle Size Analyzer (Nanotrac Particle Size Analyzer- Microtrac Nanotrac A150, Korea).

Zeta potential is the difference in the potential between the surface of tightly bound layer (shear plane) and the electroneutral region of the solution. It is measured by Malvern zeta sizer (Malvern Instruments, UK). (Martin A 1993).

Polydispersity Index

Polydispersity index is a parameter to define the particle size distribution of nanoparticles obtained from a nanotrac particle analyzer. PDI is an index of width or spread or variation within the particle size distribution. Monodisperse samples have a lower PDI value, whereas higher values of PDI indicate a wider particle size distribution and the polydisperse nature of the sample. The usual range of PDI values is; 0-0.05 (monodisperse standard), 0.05-0.08 (nearly monodisperse), 0.08-0.7 (mid range polydispersity), and >0.7 (very polydisperse) (Wolfgang S 2007).

Surface Morphology

SEM 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 (also known as “Sputter coater”) with a gold layer 20 nm thick in an argon gas environment at 45 mA current for 5 seconds. Photographs were taken using a JSM-5200 Scanning Electron Microscope (Tokyo, Japan) operated at 15 kV. (Mandal et al., 2010)

In vitro drug release

The release of drug from nanosuspension was evaluated over 24 hours by a dialysis tubing membrane (Himedia Laboratories Pvt. Ltd. India) with a molecular weight cut-off of 12,000-14,000, loaded with 10 ml of drug loaded nanosuspension and soaked in a simulated tear fluid (pH 7.4), at 37 ± 0.5°C temperature and under slow magnetic stirring. At regular intervals, aliquots of 1 ml of the dissolving medium were withdrawn, and immediately restored with the same volume of fresh fluid. The amount of drug released was assessed by measuring absorbance at 288 nm using a UV spectrophotometer (Shimadzu UV-1700 Pharmaspec, Japan) after suitable dilution. (Gupta et al., 2009).

Release Kinetics

In order to analyze 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 and Hixson Crowell models. By comparing the R² values obtained, the best fit model was selected. (Costa P et al., 2001).

Ex-Vivo Transcorneal Permeability

The transcorneal permeability of moxifloxacin from the developed formulation was studied on excised goat corneas and compared with the marketed formulation. Fresh whole eyeballs of goat were obtained from a local butcher’s shop and transported to laboratory in cold condition in normal saline. Corneas along with 5-6 mm of surrounding scleral tissue were then carefully removed and stored in freshly prepared simulated tear solution (STF), pH 7.4. The study was carried out in vertical Franz diffusion chamber where the upper chamber served as a donor compartment, in which 100 µl of drug solution formulation under study was placed. The excised goat cornea was fixed between clamped donor and receptor compartments of Franz diffusion cell in such a way that its epithelial surface faced the donor compartment. The lower chamber served as a receiver compartment which was infused with freshly prepared STF. This whole system was maintained at 37 ± 0.5°C. Samples were collected at periodic time intervals up to 8 hours in micro centrifuge tubes and subjected to the quantification of moxifloxacin by UV at 288 nm (Gupta et al., 2009).

Microbiological Studies

The microbiological studies were carried out to ascertain the antimicrobial activity of the prepared formulations and compared with marketed eye drops (MOSI® P. aeruginosa (ATCC 6580) and S. aureus (NCTC 6749) using “cup plate technique”. Muller Hinton agar medium was inoculated with the test organisms, transferred in petri plates (40 ml each) 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. Weighed quantities of nanoparticles were taken and suspended separately in normal saline solution (0.5% w/v) prior to transfer into wells. 100 µl of each of the test solution as well as marketed eye drops were placed in separate petri plate bores under aseptic conditions. Positive control (petri-plate with microorganism but placed with normal saline) and negative control (petri-plate without microorganism) were also prepared. Results were expressed as mean ± S.D (Gupta et al., 2010, Somchit MN et al., 2003).

Stability studies

Selected nanosuspension was chosen to perform short term stability studies. Samples were stored in glass vials for 3 months at 4°C in freeze and at room temperature. After 30, 60 and 90 days, samples were visually observed for any sedimentation and subjected for % EE and in vitro release studies at every one month interval.

Results and Discussion

The IR spectra of the physical mixture of both drug and polymer exhibited all the characteristic peaks as depicted in Figure 1c. Therefore, it shows compatibility of drug with the polymer.
Moxifloxacin-loaded PLGA nanoparticles were prepared using drug to polymer ratios of 1:0.1, 1:0.2, 1:0.3, 1:0.4, and 1:0.5 and evaluated for particle size, zeta potential, entrapment efficiency, in vitro release and ex vivo transcorneal permeability.

Percent Entrapment Efficiency and Percent Yield

The values of drug entrapment efficiency are shown in Table 2. The percent entrapment efficiency was found to be in the range of 27.03% to 83.1%. The maximum entrapment efficiency was found for Batch MF4 (83.1%) whereas it was less for the batch MF1 (27.03%). The EE observed was 27.03% to 83.1% with the PLGA concentration 3 to 15 mg/ml. The decreased EE of the formulation MF5 (PLGA concentration of 15 mg/ml) indicates that 12 mg/ml concentration of PLGA is optimum for obtaining maximum EE. A high copolymer concentration in the organic phase resulted in the increase in the particle size that would affect the amount of MFX adsorbed on the surface of nanoparticles.

Maximum percent practical yield was found to be 62.13% for MF-5. Percent practical yield depends upon the concentration of polymer added. It increased with the increase in concentration of polymer added to the formulation.

Particle size, Particle Size Distribution and Zeta Potential

Particle size and size distribution are very important parameters for ocular delivery purposes in order to avoid the irritation to ocular surface. The mean particle size for formulations MF1 to MF5 varied in range of 174.33 to 268.53 nm. In general, the mean hydrodynamic diameter of the particle increased with increases in polymer concentration. The increase in viscosity of the organic phase during nanoparticles preparation probably leads to a reduction in the contribution of acetone spontaneous diffusion to aqueous solutions when a higher concentration of polymer is employed. Particle size is an important parameter in the development of ocular drug delivery also to assess the ocular tolerance. Larger particle sizes will induce rapid tear production which leads to the rapid drainage of the instilled dose and therefore reduced bioavailability. All the formulations prepared showed a mean particle size below 250 nm, which is considered suitable for the ocular delivery.

The particle size distribution was narrow as the formulation has polydispersity index (PI) less than 0.1, which corresponds to a monodisperse system. The mean polydispersity index values for the drug loaded formulations varied in the range of 0.226 to 0.512 as
shown in Table 2. It could be inferred that all the formulations showed mid range polydispersity.

ζ Potential is an important parameter to analyze the long-term stability of the nanoparticles. Generally higher ζ potential values, both (+) or (−), indicate long-term stability because of electrostatic repulsions between particles with same charges avoid aggregation. The ζ potential values remained in the range of negative values for all the batches (-22.45 to -27.4 mV) (Table 2). It promotes particle stability because the repulsive forces prevent the aggregation with aging. The negative charge on the PLGA nanoparticles is due to the ionization of the carboxylic end groups of the surface polymer, as reported in the previous literature.

The optimized nanosuspension formulation was selected on the basis of small particle size, less PI and high entrapment efficiency and maximum yield. Entrapment efficiency and percent yield of the optimized formulation (MF4) were found to be 83.1% and 51.1% respectively, therefore MF4 was selected for further studies.

**Surface Morphology**

Nanoparticle surface morphology and shape were visualized using SEM (JSM-T330A, JEOL) using magnification of 15000 to 20000 X for taking photographs. The drug loaded nanoparticles of formulation MF4 was found to be spherical with a smooth surface (Figure 2).

![Fig. 2. Typical scanning electron micrograph of MFX loaded polymeric nanoparticle (MF4) with 15000 X magnification.](image)

**In vitro Release**

The profiles are biphasic, with an initial burst of drug release attributed to surface associated drug, followed by a phase of slower release as the drug entrapped inside the particle diffuses out in to the release medium. The drug release was compared with pure drug and different formulations over a 24 hour period as shown in the Figure 3.

![Fig. 3. In vitro drug release profile of pure MFX and PLGA nanoparticles MF1, MF2, MF3, MF4 and MF5.](image)
Particle size has a direct effect on the drug release profile from the formulations. Formulation MF4 with a larger average particle size 202.5 nm gave a small initial burst release of 27.68% after 2 hours and 86.1% drug release after 24 hours. It shows that smaller particles have a higher surface area compared to their volume; therefore most of the drug will be at or near the particle surface and can be readily released. Whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out.

**Kinetics of Drug Release**

Among the models tested, the drug release profiles for formulations (MF1 to MF5) were best fitted with the Higuchi Matrix model based on regression coefficients 0.9831, 0.9911, 0.9810, 0.9756 and 0.9840, respectively. The linearity of the plot indicated that the release process was diffusion-controlled. Thus, the amount of drug released was dependent on the matrix drug load. The diffusion exponent (n) values for all formulations were less than 0.5, indicative of a non-fickian mechanism of drug release.

**Ex vivo Transcorneal Permeability**

Results indicated that the inclusion of MFX in the colloidal system considerably increased the penetration rate of the drug across the cornea. Moxifloxacin from marketed formulation permeated 36.9% in 4 hours whereas moxifloxacin from PLGA nanoparticles (MF4) permeated 47.43% in 4 hours.

The ability of substances to diffuse through epithelial barriers depends not only on the properties involved but also on the chemical nature, size and conformation, lipid/water partition coefficient, and degree of ionization of the permeant molecules tested etc. For molecular diffusion studies, the cornea may be considered as consisting three primary layers (i.e epithelium, stroma and endothelium). Depending on the physico chemical properties of the permeant, the diffusional resistance offered by the individual layers can vary greatly. Epithelium which is lipid in nature and consists of roughly five layers of epithelial cells is the main barrier, offering high resistance to the diffusion of ionic or relatively hydrophobic agents. The aqueous stroma, which constitutes the bulk of the cornea both in mass and thickness, is the major rate-limiting barrier to the diffusion of hydrophobic agents.

MFX-loaded PLGA nanoparticles showed a significantly higher drug permeation capability compared with the commercial marketed eye drops. This favorable penetration of MFX across the cornea could be attributed to the agglomeration of the nanoparticles in the conjunctival sac, thus forming depot from which the drug is slowly delivered to the precorneal area.

**Microbiological Studies**

Formulation MF4 and marketed eye drops (MOSI® eye drops) of moxifloxacin HCl were evaluated for antimicrobial activity by the cup-plate method. All formulations gave a clear zone of inhibition comparable with the zone of inhibition given by marketed eye drops (Figure 5 and Table 3). The diameter of the zone of inhibition was 34.3 ± 2.07 cm and 28.7 ± 3.42 cm after 24 hours for the marketed eye drop and formulation MF4 respectively. Results showed that formulation MF4 has better antimicrobial efficacy compared with marketed eye drops. Results obtained were compared with the control (without drug).

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Antimicrobial activity of optimized nanosuspension (MF4) and marketed product against P. aeruginosa and S. aureus.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation</td>
<td>Zone of Inhibition (mm)</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Marketed Eye Drops</td>
<td>27.2</td>
</tr>
<tr>
<td>MF4</td>
<td>32.1</td>
</tr>
<tr>
<td>Positive Control</td>
<td>0</td>
</tr>
</tbody>
</table>

**Fig. 4.** Comparative ex vivo transcorneal permeability of formulation MF4 with marketed conventional formulation.
Stability Studies

Many factors including the stability of the active ingredient(s); the potential interaction between active and inactive ingredients; the manufacturing process; the dosage form; and handling, etc. affect the stability. The physical appearance of the MF4 nanosuspension changed slightly when samples were stored at 4 ± 1°C for 3 months. A thin layer of sediment was observed when nanosuspension was stored at 4 ± 1°C for 3 months. However, it disappeared immediately with slight shaking. There were negligible changes in the initial values of % entrapment efficiency and in vitro release values after 3 months. Thus, 4 ± 1°C was found to be optimum condition for storage of prepared nanoparticles.

Conclusion

From the above findings, it can be concluded that PLGA can be successfully used to design sustained release nanoparticle formulations for ophthalmic drug delivery in the treatment of bacterial conjunctivitis. Among different formulations prepared by nanoprecipitation method formulation, MF4 with drug to polymer ratio (1:0.4), showed satisfactory results; i.e mean particle size of 202.5 nm (majority of particles were in the range of 174.33 to 268.53 nm), polydispersity index of 0.226, zeta potential of −25.45 mV and percent yield of 51.81% and entrapment efficiency of 83.1%. The formulation MF4 has better antimicrobial efficacy than marketed eye drops.

Future work to be taken up with respect to in vivo pharmacokinetics and pharmacodynamics and long term stability studies performed in order to characterize the delivery system for clinical use.

References


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