ABSTRACT
Moxifloxacin (MOX) is a fluoroquinolone anti-infective drug, indicated for the treatment of bacterial conjunctivitis. The drug is soluble in water but still produces low ocular bioavailability due to biological barriers and so it requires dosing for two/three times a day. The present study was designed to formulate, optimize and characterize polymeric Nanoparticles MOX for ocular administration using Ion Exchange Resin (IER). IER-nanoparticles were prepared by media milling method, formulation/process parameters were optimized based on evaluation parameters such as color of nanosuspension, sedimentation behaviour, particle size and zeta potential. MOX-IER nanosuspensions were prepared at different stoichiometric ratio of MOX and IER and characterized by entrapment efficiency, pH, particle size and zeta potential of nanosuspension. In vitro release study of optimized batch MNIER3 exhibited sustained release pattern which follows Korsmeyer-Peppas model with Fickian diffusion mechanism for drug release. Based on these results optimized batch of MOX-IER nanosuspension formulated in the laboratory was found suitable for ocular delivery.

Keywords: Moxifloxacin; nanoparticles; nanosuspension; media milling; stoichiometric ratio; sedimentation behaviour.

INTRODUCTION
Infectious diseases are one of the strongest reasons of deaths in the world due to antibiotic resistance and chemical limitations on synthesis of the new antibiotic molecules. Bacterial conjunctivitis which is prevalent in patients of all ages is a common health-care issue for the general practitioner and the ophthalmologist. It is an inflammation of the conjunctiva caused by bacteria. In majority clinical cases the cause is Staphylococcus, Streptococcus, Haemophilus and Moraxella strains. Typical symptoms include red eye/ pink eye, mucous and pus discharge persistent throughout the day, pain and itching. Bacterial conjunctivitis can be contracted directly from infected individuals or can result from abnormal proliferation of the native conjunctival flora. Pharamacological treatments of Bacterial conjunctivitis include Aminoglycosides, Bacitracin, Polymyxin B Combinations, Fluoroquinolones and Macrolide antibiotics in the ascending order of choice. In general Aminoglycosides are used as first line of treatment and Macrolide antibiotics are considered as the last line of treatment.

Moxifloxacin is a 4th generation fluoroquinolone anti-infective indicated for the treatment of bacterial conjunctivitis with good ocular penetration and potency against gram positive bacteria. Moxifloxacin is soluble in water. Due to various biological barriers in Ocular drug delivery like (a) drug dilution on the precorneal tear film and rapid renewal rate of lachrymal fluid and blinking reflex (b) systemic drug absorption (c) rapid drug drainage from the site of application (d) lacrimation (e) aqueous humor drainage (f) corneal diffusion barrier, topically administered drugs are reaching in low concentration to the target site in the eye. Water soluble drugs are rapidly drained from the site of action. The unmet need is to develop topical formulation with sustained drug release effect which ultimately reduced dosage frequency also.

Colloidal drug carriers have significant technological and therapeutic advantages in ophthalmic therapy with respect to conventional aqueous eye drops like increase in precorneal drug retention time, sustained drug release,
reduction in administration frequency, reduced drug toxicity, and targeted delivery to specific eye tissues [12,13,14,15]. Polymeric nanoparticles are one of the promising formulations for ophthalmic delivery using various polymers such as Ethyl cellulose and Eudragit [16], Chitosan, Sodium alginate, Poly-e-caprolactone, Poly-L-lactide, and Poly-D, L-lactide-co-glycolide [17,18]. Nanoparticles form cross-linked collagen shields have been prepared and characterized for sustained delivery of drug after topical ophthalmic application [20]. Lipid nanoparticles [21], Lipid-polymer hybrid nanoparticles [22], Human serum albumin nanoparticles [23] have also been studied. Liposomes [24,25] and Microemulsions [26] have been developed for ophthalmic delivery.

In current study, nanoparticles using Ion Exchange Resin (IER) were prepared. IER are polymers containing appropriately substituted acidic groups, such as carboxylic and sulfonic for cation exchangers; or basic groups, such as quaternary ammonium group for anion exchangers. IER forms complex with acidic and basic both type of polymer because they are in ionized form. Drug-IER complexes are insoluble in water and tend to release the drug from exchange of bound drug ions by ions normally present in body fluids [27]. Traditionally IER is utilized for taste masking and sustained drug release in oral formulations [29,30,31,32,33]. IER is enormously used in variety of drug delivery systems such as adhesive in conjugation with nanoparticles for subcutaneous delivery [35], dental adhesive [36] and ocular sustained drug release [37]. Drug-IER complex suspension is approved and commercially available in US market for ophthalmic delivery as Betoptic S®[38].

Aim of the present study is formulation of Moxifloxacin-IER complex nanoparticles, optimization of (formulation and process) parameters and characterization of nanosuspension followed by evaluation of In vitro drug release behaviour to assess the scope of reducing the dosage frequency. Materials and Methods

MATERIALS AND METHODS

Moxifloxacin Hydrochloride was obtained as gratis sample from Torrent Pharmaceuticals Ltd. (Ahmedabad, India). Sodium polystyrene sulfonate, Ion Exchange Resin (IER) was a gift from DOW Chemicals (Mumbai, India). Yttrium stabilized Zirconium beads (Zirmil® Y, 0.3 mm and 0.8 mm) were received as a kind gift from Saint Gobain (Magnus Impex Private Ltd., New Delhi, India). Cerium stabilized Zirconium beads (Zirconox®, 0.7-1.2 mm & 1.2-1.7 mm) were purchased from Jyoti Ceramic (Nashik, India). Dialysis membrane (12000 M.W.C.O., DM-70) was obtained as a gift from Hi Media (Mumbai, India). All other chemicals like Sodium chloride, Sodium bicarbonate and Calcium chloride were of analytical grade. Freshly prepared distilled water was used to prepare formulations. Sieve of 250 micron, ASTM Mesh # 60 was purchased from Wire Metal GMP Products, Mumbai, India.

Drug characterization

Solubility: According to Literature [39], Moxifloxacin Hydrochloride is soluble in water. Solubility study was performed by dissolving one unit of drug in one unit of solvent. Initially 1 mg of drug was dissolved in 1 ml of water at room temperature, 20-25°C. After this, if drug was completely dissolved, then more amount of drug was added. If not so, then more amount of solvent is added. Solution was then kept for 24 h to check re-predipitation at room temperature, 20-25°C[40].

Spectrophotometric analysis: Solution of Moxifloxacin Hydrochloride prepared in distilled water was run in UV Visible Spectrophotometer (Shimadzu, UV-1700 PharmaSpec, Kyoto, Japan) from 200 to 400 nm to estimate wavelength at which maximum drug absorbance is noted. At maximum wavelength Standard Calibration Curve was prepared by preparing drug solutions of suitable concentrations in distilled water.

Drug-IER compatibility

Physical mixture of Drug and IER in 1:1 stoichiometric ratio was prepared and MOX content was estimated after incubation of 1 month accelerated stability at 40°C ± 2°C and 75 %RH ± 5 %RH in accordance with International conference on harmonization (ICH) guidelines, Q1A-R2 in stability chamber (Durga Scientific Pvt. Ltd., Vadodara, India). Physical mixture powder was suspended in distilled water and solubilized drug was filtered through Whatman filter paper and estimated by UV Spectroscopy at 289 nm against distilled water as reference.

Formulation of IER nanoparticles

Ion Exchange Resin (IER) nanoparticles were prepared by Top down technique, wet milling[41,42,43,44,45,46,47]. Two types of Zirconium beads were used for the experiment: a) Cerium stabilized and b) Yttrium stabilized. IER suspension in distilled water was prepared and milled in a plastic bottle with the Zirconium beads on magnetic stirrer (Bio-Lab, Magnetic stirrer with hot plate, BL-223B, Vadodara, India) using teflon coated stir bar. Milling process parameters of all the IER nanoparticles batches are presented in Table 1. Batch size of IER nanoparticles was kept constant, 25 ml. At laboratory scale, milling time was fixed to 1 week (168 hours) for all batches until all the process parameters were selected. Milling was carried out at room temperature, 20-25°C. Volume based ratio of Zirconium beads quantity was used for IER milling. [Example of calculation: Bead quantity used for 25 ml batch size: 70% volume of Ce stabilized Zr beads = 12.5 ml = 68 gm (bulk density:3.9 gm/cc)]. After achieving nano size, IER nanoparticles, 25 ml (approx. 20% w/v concentration) were washed with distilled water to separate from Zirconium beads using 250 micron sieve [ASTM Mesh #60] and volume was made up to 100 ml to prepare approx. 5% w/v IER suspension. The IER nanoparticles suspension was stored in tightly closed containers for further characterization.

Characterization of IER nanoparticles

Description: IER nanoparticles were collected to in transparent glass vials. Colour and physical appearance of samples of IER nanoparticles batch were noted by visual observation.
Sedimentation behaviour: Sedimentation behaviour was assessed by Sedimentation photograph capturing method over the period of specified time points\(^{[48,49,50]}\). IER nanoparticles batches after completion of specified milling time (Table 1) were placed in transparent glass vials and photographs of sediment and supernatant were captured after 8, 24 and 48 hours. Captured images of these time points were compared with photographs of 0 hours and after 48 hours. Captured images of these time points and photographs of sediment and supernatant were captured over the period of specified time points.

**Particle size and Polydispersity Index (PDI):** The batch of optimized IER nanoparticles was estimated for particle size and Polydispersity Index (PDI) using Dynamic Light Scattering by Malvern Zeta sizer (Malvern Instruments Ltd, NanoZS, UK) after dilution with distilled water (1:1000).

**Zeta potential:** The Zeta potential of batch of optimized IER nanoparticles was determined using Dynamic Light Scattering using Malvern Zetasizer after dilution with distilled water (1:1000). The sample was taken into small disposable zeta cell by taking care to avoid air bubble entrapment leading to the correct measurements.

**Formulation of MOX-IER nanoparticles**

Moxifloxacin Hydrochloride (MOX) and IER complex was prepared in various stoichiometric ratio (Table 2). Briefly, MOX was dissolved in distilled water at room temperature, 20-25°C and added to IER suspension under stirring on Magnetic stirrer and stirring was continued for 30 min. Volume make was done up to batch size, 50 ml with distilled water.

### Table 1: Effect of various milling process parameters on IER nanoparticles batches

| Batch no. | IER (%) | Type of Zr beads | Zr bead size (mm) | Zr bead volume (%) | Milling time (hours) | Description | Sedimentation behaviour After 48 hours |
|-----------|---------|------------------|-------------------|--------------------|----------------------|-------------|-------------------------------------|
| NIER1     | 30      | Ge stabilized    | 1.2-1.7           | 70                 | *                    | Brown Susp. | Not applicable                      |
| NIER2     | 30      | Ge stabilized    | 1.2-1.7           | 70                 | *                    | Brown lumps | Not applicable                      |
| NIER3     | 20      | Ge stabilized    | 1.2-1.7           | 70                 | 168                  | CreamSusp.  | SedP                                |
| NIER4     | 20      | Ge stabilized    | 0.7-1.2           | 70                 | 168                  | CreamSusp.  | SedP                                |
| NIER5     | 20      | Y stabilized     | 0.8              | 70                 | 168                  | CreamSusp.  | SedP                                |
| NIER6     | 20      | Y stabilized     | 0.8              | 40                 | 168                  | CreamSusp.  | SedP                                |
| NIER7     | 20      | Y stabilized     | 0.8              | 90                 | 168                  | CreamSusp.  | SedP                                |
| NIER8     | 20      | Y stabilized     | 0.3              | 40                 | 168                  | CreamSusp.  | SedP                                |
| NIER9     | 20      | Y stabilized     | 0.8              | 40                 | 72                   | CreamSusp.  | Not applicable                      |
| NIER10    | 20      | Y stabilized     | 0.3              | 40                 | 96                   | CreamSusp.  | Non SedP                            |
| NIER11    | 20      | Y stabilized     | 0.8              | 40                 | 48                   | CreamSusp.  | Not applicable                      |
| NIER12    | 20      | Y stabilized     | 0.3              | 40                 | 48                   | CreamSusp.  | Not applicable                      |
| NIER13    | 20      | Y stabilized     | 0.8              | 40                 | 48                   | CreamSusp.  | Not applicable                      |
| NIER14    | 20      | Y stabilized     | 0.3              | 40                 | 48                   | CreamSusp.  | Not applicable                      |

*Inadequate Milling; SedP- Sedimenting Particles; Non SedP- Non Sedimenting Particles

### Table 2: Composition of MOX-IER nanoparticles

| Batch     | Drug: Resin Ratio | MOX (% w/w) | IER (% w/w) |
|-----------|-------------------|-------------|-------------|
| MNIER1    | 1:0.2             | 0.5         | 0.1         |
| MNIER2    | 1:0.5             | 0.5         | 0.25        |
| MNIER3    | 1:1               | 0.5         | 0.5         |
| MNIER4    | 1:1.5             | 0.5         | 0.75        |
| MNIER5    | 1:2               | 0.5         | 1.0         |

Batch size - 50 ml
Characterization of MOX-IER nanoparticles

Description: Samples of MOX-IER nanoparticles suspension were collected in transparent glass vials and colour and physical appearance were evaluated by visual observation.

Entrapment efficiency: The amount of drug entrapped into IER nanoparticles was calculated using indirect method, by measuring unbound drug concentration\[55\], Supernatant obtained after ultracentrifugation (Bio-Lab, Micro Centrifuge, BL-135, India) of MOX-IER nanoparticles at 10,000 rpm for 75 min were filtered with Watman filter paper to get clear solution and the same was analyzed by UV spectroscopy at 289 nm against distilled water.\% Entrapment efficiency was calculated as per below equation.

\[
\text{Entrapment Efficiency (\%)} = \frac{\text{Initial drug} - \text{Free drug}}{\text{Initial drug}} \times 100 \quad (1)
\]

pH: 5 ml of suspension was taken in a 10ml beaker and pH was recorded using calibrated pH meter (Chemiline, Digital pH meter, CL-110, London, UK) by bringing a glass electrode near the surface of the formulation and allowing the equilibration for 1 min.

Particle size and Polydispersity Index (PDI): The optimized batch of MOX-IER nanoparticles was estimated for particle size and Polydispersity Index (PDI) using Dynamic Light Scattering by Malvern Zeta sizer after dilution with distilled water (1:100).

Zeta potential: The Zeta Potential of optimized batch of MOX-IER nanoparticles batch was determined using Dynamic Light Scattering using Malvern Zetasizer after dilution with distilled water (1:100). The sample was taken into small disposable zeta cell by taking care to avoid air bubble entrapment leading to the correct measurements.

In vitro release studies: In vitro release study of MOX-IER nanoparticles and MOX solution as control was executed by dialysis bag method\[56\]. Freshly prepared Simulated tear fluid (STF) containing 0.67 g of Sodium chloride, 0.20 g of Sodium bicarbonate, 0.006 g of Calcium chloride dihydrate and 100 mL of deionised water\[56\] was used as dissolution medium. 0.1 g of MOX-IER nanosuspension was suspended in 12,000 kDa molecular weight cut-off dialysis membrane (DM-70, Hi Media, Mumbai, India) after activation in alkaline solution. Both ends of the dialysis membrane were tied with thread and put in a beaker containing 100 mL dissolution media under stirring at 75 rpm on magnetic stirrer at 37 ± 0.5°C. At specified time points, 1 mL of sample was withdrawn and sink condition was maintained with equal amount of fresh dissolution media. Withdrawn samples were centrifuged at 10,000 rpm for 75 min and then filtered with Watman filter. The concentration of MOX was analysed in filtered samples by UV spectroscopy at 289 nm. Similary In vitro release of drug in distilled water was evaluated using same experimental conditions and time intervals.

Statistical analysis: In vitro release study was performed for 12 dosage units for each time point for validity of statistical analysis. Results are expressed as mean ± SEM. Two sample t-test was applied to analyse In vitro drug release data at 95\% Confidence Interval (CI) using Minitab® 18.1 (Minitab Inc., Pennsylvania, USA).

RESULTS AND DISCUSSION

Drug characterization

Solubility: Solubility of Moxifloxacin Hydrochloride was found to be 1 g in 60 ml distilled water (approx. 16 mg/ml) at room temperature, 20-25°C which is sparingly soluble as per USP solubility classification, 1 part in 30 to 100 parts of solvent.

Spectrophotometric analysis: Maximum absorbance of 0.818 was noted at maximum wavelength 289 nm in distilled water which is supported by literature\[54\]. At 289 nm, linear curve of 2, 4, 6, 8, 10 ppm Moxifloxacin Hydrochloride solution in distilled water was prepared with regression coefficient, \( r^2 \) of 0.992 (calculated using Microsoft Excel, Windows® 2013) reflecting linearity of the plot. From the calculated linear equation \( y = mx + c \), concentration of MOX in all the samples was determined.

Drug-IER Compatibility

No significant difference was noted in MOX content in the physical mixture of Drug and IER before and after incubation of 1 month accelerated stability, 99.47\% w/v and 98.62\% w/v respectively.

Formulation of IER nanoparticles

Apparently during preparation of nanoparticles, as the particle size is decreased change in colour is observed e.g. gold nanoparticles change their colour from blue to red\[55\]. Studies also have been carried out to observe effect of particle size reduction on optical properties and colour change of pigments prepared by grinding\[56\]. Nano sized particles merely do not settle in the dispersion and micron sized particles settle quickly with respect to time\[57\]. Milling process parameters for preparation of IER nanoparticles were optimized considering these two characterizations: (a) Description: colour change (brown to cream) (b) Sedimentation behaviour: non sedimenting particles. (Table 1). Milling process parameters were for optimized based on OFAT approach, One Factor at a Time\[58\]. Suitable container for milling was found to be HDPE bottle considering inefficient milling in LDPE bottle because the latter was unable to bear the load of highly dense Zr beads (NIER1). 20\% concentration of IER at laboratory scale of 25 ml was found to be appropriate considering lumps formation at higher concentration and feasibility (NIER2 and NIER3). Since particle size reduction was observed in similar fashion with Yttrium stabilized Zr beads and Cerium stabilized Zr beads considering higher mechanical strength of former, Yttrium stabilized Zr beads were selected for further milling batches (NIER3, NIER4 and NIER5). Yttrium stabilized Zr beads are most inert media used in pharmaceuticals as they do not interact with drug and avoid incorporation of impurities in the formulation\[59\]. Since there was no significant change in description of samples of IER nanoparticles at various bead volume ratios, 40\% bead volume was selected for further milling batches (NIER5, NIER6, NIER7).

Bead size selection was done with the support of extensive literature and characterization of IER nanoparticles batches. For instance, various IER nanoparticles batches were prepared by keeping aforementioned selected process parameters constant and varying bead size & combinations. As per literature pertaining to milling technology\[50,61,62\] bead size should be approximately 10x smaller than input material particle size or approximately 1000x larger than desired particle size. So, for IER milling, ideal bead sizes can be 0.5 mm (considering target desired nano size 500 nm) or 0.75 mm to 1.5 mm [considering IER input particle size 75-150 micron (Product Data sheet, 2016)]. Preparing IER nanoparticles batches using bead size in the mentioned range (NIER1 to NIER8) showed sedimenting particles in the samples, hence could not achieve nano sized particles. The particle size reduction effect could be enhanced by using
smaller bead size\cite{63}. Therefore combination approach was tried using two sized beads: milling of IER suspension by larger sized beads (0.8 mm) followed by smaller sized beads (0.3 mm) which yielded nanosized particles (NIER9). Then after efforts were made to reduce milling time with combined bead sizes and as a result, nanosized particles were obtained after 96 hours evident by two consistent batches with non sedimenting particles (NIER11 and NIER12) and particle size reports of optimized batch (NIER13).

**Characterization of IER nanoparticles**

**Description:** Colour and physical appearance of the IER samples was found to be brown and suspension form when particle size is not reduced and the same was cream suspension for the samples where particle size was reduced from raw resin (Table 1).

![Figure 1: Sedimentation behaviour: Left vial, Control – Unmilled IER Susp., Right vial – NIER8, Sedimenting particles in IER Susp.](image1)

Sedimentation behaviour: Sedimentation behaviour of all IER nanoparticles batches is presented in Table 1. Literature reveals that suspended particles in the dispersion with higher particle size settle at higher rate than that of lower particle size\cite{64}. The same was evident in current experiment. IER nanoparticles with higher particle size settled significantly (Figure 1, representative batch NIER8) and nanosized particles did not settle (Figure 2, representative batch NIER9) over the period of 48 hours when compared with unmilled Ion Exchange Resin suspension.

![Figure 2: Sedimentation behaviour: Left vial, Control – Unmilled IER Susp., Right vial – NIER9, Non sedimenting particles in IER Susp.](image2)

**Particle size and Polydispersity Index (PDI):** Particle size of optimized IER nanoparticles batch, NIER13 was found to be 360 nm (Figure 3) reflecting smaller nanosized particles. Lesser PDI i.e. 0.221 proved that prepared nanoparticles have uniform and narrow size distribution\cite{65}.
Figure 3: Particle size distribution study of optimized IER nanoparticles batch NIER13

Zeta potential: Nanoparticle surface charge (Zeta Potential) of optimized batch, NIER13 (Figure 4) was estimated to -41.3 mV reflecting good physical stability of the suspension. Higher value of Zeta potential in positive or negative side preferably above +30 mV or -30 mV indicates stable nanosuspension when kept in storage for long time because of very low or no chances of aggregation between the nanosized particles. Negative values of Zeta potential reflects anionic chemical nature of IER, Sodium polystyrene sulfonate due to presence of SO₃⁻ functional group.

Figure 4: Zeta potential study for optimized IER nanoparticles batch NIER13
Formulation of MOX-IER nanoparticles

Moxifloxacin bears cationic charge due to presence of tertiary amine functional groups. Sodium polystyrene sulfonate which is a strong cation exchange resin bears anionic charge due to presence of sulfonate functional group on to styrene divinyl benzene backbone. Both form an ionic complex. Structures are presented in Figure 5 (a,b). MOX and IER stoichiometric ratio was decided considering the results of Entrapment efficiency.

Characterization of MOX-IER nanoparticles

Description: Colour and physical appearance of the MOX-IER nanoparticles samples was found to be cream suspension.

Entrapment Efficiency: MOX-IER stoichiometric ratio optimization was critically carried out on the basis of Entrapment Efficiency. From the Graphical presentation (Figure 6) it was observed that MOX-IER ratio was inversely proportional to %EE up to definite concentration of resin. Based on Entrapment Efficiency results 81% to 95%, highest EE was achieved with Drug resin ratio 1:1, 1:1.5 and 1:2. Amongst 1:1 Drug resin ratio was chosen with the fact that highest EE could be achieved without using very high concentration of IER.

pH: pH of Moxifloxacin HCl was 4.66 reflecting cationic nature of the drug. pH of MOX-IER nanoparticles was in the range of 5.6 to 6.0. The same was measured to be 5.74 the optimized batch, MNIER3 which is moving towards neutral reflecting ionic complexation from drug solution pH.

Particle size and Polydispersity Index (PDI): Particle size distribution result (Figure 7) of MNIER3, 301 nm and lesser PDI i.e. 0.215 was an evident that Resin nanoparticles remained non aggregated uniform suspended particles after Moxifloxacin entrapment.
Zeta potential

**Results**

| Z-Average (d.nm) | % Intensity | St Dev (d.nm) |
|------------------|-------------|--------------|
| 301.1            | 100.0       | 197.9        |

Peak 1: 382.9

Peak 2: 0.000

Peak 3: 0.000

**Result quality:** Good

**Figure 7:** Particle size distribution study of optimized MOX-IER nanoparticles batch MNIER3

**Zeta Potential:** Nanoparticle surface charge of Drug loaded Resin Nanoparticles optimized batch, MNIER3 was estimated to -37.8 mV (Figure 8) reflects that the suspension retained physical stability after entrapment with Moxifloxacin.

**In vitro release studies:** Cumulative drug release (CDR) of MOX was compared as a mean of 12 units of MOX-IER nanoparticles and MOX aqueous solution (Figure 9). MOX was released faster in aqueous solution than nanoparticles form. Approx. 95% of drug was released from MOX aqueous solution in just 1 hour and the same took 4 hours from MOX-IER nanoparticles which apparently indicate the sustained release of Moxifloxacin from drug-resin complex (MOX-IER) nanoparticles.

**Results**

| Zeta Potential (mV) | Peak 1 | Area (%) | St Dev (mV) |
|----------------------|--------|----------|-------------|
| -37.8                | -39.6  | 85.8     | 7.23        |

Peak 2: -22.1

Peak 3: 0.00

**Result quality:** See result quality report

**Figure 8:** Zeta potential study of optimized MOX-IER nanoparticles batch MNIER3
Various mathematical models were applied to understand drug release kinetics like Zero-order, First order, Higuchi and Korsmeyer–Peppas\(^{68,69}\). Graphical presentation is depicted in Figure 10 and linear regression analysis is summarized in Table 3. Based on Correlation Coefficient (\(r^2\)) value 0.912, Korsmeyer-Peppas model was considered to be the best fit model MOX-IER nanoparticles.

The value of release exponent \(n\) in Korsmeyer-Peppas model indicate mechanism of drug release: 0.5 indicates Fickian diffusion, between 0.45 and 0.89 indicates Non-Fickian transport, 0.89 indicates Zero order release and \(n\) value higher than 0.89 indicates Super case II transport mechanism. \(n\) value of 0.4387 for MOX-IER nanoparticles reveals that drug release is diffusion controlled (Fickian diffusion).

### Statistical analysis

| Drug release Kinetics model | Equation   | \(r^2\) |
|----------------------------|------------|---------|
| Zero-order                 | \(y = 8.9517x + 42.554\) | 0.692   |
| First order                | \(y = 0.0691x + 1.5717\)  | 0.550   |
| Higuchi                    | \(y = 32.741x + 39.712\)  | 0.851   |
| Korsmeyer-Peppas           | \(y = 1.6908x^{0.4387}\) | 0.912   |
Significant difference in % CDR at each time point was observed based on two sample t-test results at p value <0.05 using Minitab® 18.1 between MOX aqueous solution and MOX-IER nanoparticles.

CONCLUSION

Developed MOX-IER nanoparticles in form of nanosuspension was a physically stable formulation with uniform particle size distribution providing high entrapment efficiency and showed sustained drug release behaviour in simulated tear fluid. These qualities make this formulation suitable for ocular delivery system.

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Declaration of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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