Formulation and Evaluation of Moxifloxacin Hydrochloride Niosomes for Controlled Ophthalmic Drug Delivery

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Abstract   The objective of present invesigation was to formulate and evaluate a niosomal delivery system of moxifloxacin hydrchloride for the treatment of ocular infections. Moxifloxacin-loaded niosomes were prepared by using thin film hydration technique and were investigated for surface pH, morphology, entrapment, in-vitro release, TEM (transmission electron microscopy), physical stability & ocular irritancy test. The release study profile was subjected to release kinetics models. All the vesicles were uniform and spherical in size. The drug relaese pattern of all formulation follows decreasing order: MN3 > MN6 > MN9 > MN5 > MN2 > MN1 > MN8 > MN4 > MN7. The formulation MN3 (span 60: cholesterol) molar ratio produce faster release of drug i.e. 77.98% after 12 hours, concluded less sustained action. The study concluded that the moxifloxacin loaded niosomes to be effective in sustaining the drug release leading to decreased side effects and increased patient compliance

Keyword: Moxifoxacin, Niosomes, Film Hydration Technique, sustianed release.

1. INTRODUCTION

Eye is the most simply accessible site for topical administration of a medication. Drugs are commonly applied to the ocular system for a localized action on the surface or in the interior of the eye (Malviya *et al.*, 2011). Ocular

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drug delivery has been a foremost challenge for researchers because of the distinctive anatomy and physiology of eye which contains different barriers such as different layers of cornea, sclera and retina in addition to blood retinal barriers, lachrymal fluid-eye barrier and also drug loss from the ocular surface. The major challenge to the formulator is to outwit these barriers without causing any tissue damage (Tangri *et al.*, 2011). The cornea is the anterior layer of the eye, comprises of epithelium, stroma and endothelium. However, this layer represents a mechanical barrier restraining the delivery of drug molecules. Due to their high lipid content, the epithelium and the endothelium are considered as an obstruction to the passage of hydrophilic molecules. The stroma is characterized by a high water content that makes this layer impermeable to lipophilic molecules (Achouri *et al.*, 2013). Corneal barrier also plays a considerable role in low ocular bioavailability due to which only < 5% of the applied drugs are able to penetrate though the cornea into the intra-ocular tissues (Maurice, 1984). Inconvenience in using conventional dosage forms are as follows:

- Limited permeability which leads to lower absorption.
- Rapid elimination.
- Frequent instillation.
- Lower bioavailability due to low precorneal residence time.
- Drainage of drugs from nasolacrimal duct (Kumar, 2012; Patel, 2010)

The conventional ophthalmic dosage forms suffer from many disadvantages including precorneal loss due to tear dynamics, insufficient residence time in the conjunctival sac and non-productive absorption. In the formulation of ophthalmic dosage forms, great attention is now being devoted to new drug delivery systems which can ensure a localised effect over prolonged time duration and increase the corneal permeability of poorly permeable drugs. To achieve these objectives *i.e.* to increase the corneal residence time, to obtain maximum corneal permeability and sustained drug release, several vesicular systems have been investigated (Shell, 1982; Habib, 2010; Aggarwal, 2004). These all systems have some drawbacks like unable to control the drug permeation through cornea; consequently, the drug concentration at the site of action might remain inadequate.

To overcome these problems, vesicular systems have been prepared to increase corneal permeation by formulating a lipid complex and enhance the percent permeation by a controlling manner. Niosomes are non-ionic surfactant vesicles and like liposomes, are bi layered structures, which can entrap both hydrophilic as well as lipophilic drugs, either in the aqueous layer or in lipid vesicular membranes (Aggarwal, 2005).

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Niosomes in topical ocular delivery are preferred over other vesicular systems because they are chemically stable as compared to liposomes due to the presence of negative charges, have low toxicity because of their non-ionic nature; unlike phospholipid, require no special precautions in handling and exhibit better flexibility in their structure (Carafa, 1998). Moreover, non-ionic surfactant vesicles (niosomes) may promote drug absorption by preferentially modifying the permeability characteristics of the conjunctival and scleral membranes.

Moxifloxacin is a fourth generation fluoroquinolone with methoxy group in C-8 position and a bulky C-7 side chain (Yoshioka, 1994). Like other fluoroquinolones, moxifloxacin (0.5%w/v) eye drops require 1-2 drops administered 6 times daily or more in severe conditions.The bactericidal activity of moxifloxacin is mediated by the inhibition of DNA gyrase (topoisomerase II) and topoisomerase IV, essential enzymes involved in bacterial DNA replication, transcription, repair, and recombination (Pestova, 2000). Moxifloxacin is more effective than ciprofloxacin or levofloxacin in experimental keratitis in rabbits (Dajcs, 2004). Penetration of moxifloxacin into the inflamed ocular tissue of rabbits has been found to be better than ciprofloxacin, lomefloxacin, ofloxacin, or levofloxacin (Yagci, 2007).

The niosomal drug delivery has been known to prolong the corneal residence time and maintain drug concentration in vitreous humor and aqueous humor by penetrating the corneal barrier i.e. in sustained manner. Recent vesicular systems have shown significant drug level in aqueous humor. Niosomal drug delivery in ophthalmic medication also showed improved transcorneal permeation of many drugs over conventional ophthalmic solutions (Hardman, 2001; Kaur, 2010; Abdelkader, 2011).

2. MATERIALS AND METHODS

2.1 Materials

Moxifloxacin hydrochloride was received from Ind-Swift Laboratories Limited, Derabassi, Punjab, India as a gift sample. Cholesterol and Span 60 were purchased from Loba Chemie, Mumbai. Dicetyl Phosphate (DCP) was procured from Sigma-Aldrich Pvt. Ltd. Mumbai, India. Dialysis memberane, 12000-14000 molecular weight cut off was obtained from Himedia Laboratories Pvt Ltd India. All other reagents used were of analytical grade.

2.2 Method of preparation of moxifloxacin hydrochloride niosomes

Non-ionic surfactant vesicles containing Moxifloxacin hydrochloride were prepared by thin film hydration technique (table 1). Briefly mixtures of

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Table 1: Composition of Moxifloxacin Hydrochloride Niosomes.

Formulation Code	Drug (mg)	Span 60 (μmol)	Cholesterol (μmol)	DCP (%)
MN1	10	150	150	2.5
MN2	10	175	150	2.5
MN3	10	200	150	2.5
MN4	10	150	150	5.0
MN5	10	175	150	5.0
MN6	10	200	150	5.0
MN7	10	150	150	7.5
MN8	10	175	150	7.5
MN9	10	200	150	7.5

surfactant (span 60), Cholesterol, were accurately measured into a long necked quick fit round bottom flask and dissolved in 10 ml of a chloroform / mixture (1:1, v/v). To the above mixture, dicetyl phosphate (DCP) was added in different concentrations i.e. 2.5%, 5% & 7.5%. The organic solvents were slowly evaporated under pressure, using a rotary evaporated (Model RVO5-ST, Perfit Laboratories, Chandigarh, India) at 60°C such that a thin dry film of the components was formed on the inner wall of the rotating flask. The film was re dissolved in 10ml of phosphate buffer saline (pH 7.4) solution containing 10 mg drug was added. The mixture was sonicated for 2 min using ultra sonicator. The resultant opalescent dispersion was rotary evaporated to disrupt the gel formed immediately. Following addition of 10 ml phosphate buffered saline (pH 7.4); rotary evaporation was continued for additional 15 min duration to ensure the removal of residual organic solvents. The niosomal suspension was left to mature overnight at 4°C (Patel *et al*., 2011).

2.3 Evaluation parameters for moxifloxacin hydrochloride niosomes

2.3.1 Determination of pH

The ophthalmic formulations should have a pH 6.6-9.0 to avoid irritation. pH of the niosomal formulations were determined using pH meter (Digital Systronics, Mumbai, India).

2.3.2 Determination of moxifloxacin hydrochloride entrapment efficiency (%EE)

The portion of the encapsulated moxifloxacin hydrochloride was obtained by ultra-centrifugation 1ml of the niosomal suspension at 18000 rpm for 1hour using a cooling centrifuge at 4°C (REMI, India). The supernatant was removed and the formed niosomal pellets were re-suspended in phosphate buffer saline (PBS) pH 7.4 to ensure the complete removal of all free moxifloxacin hydrochloride. The supernatant (free moxifloxacin hydrochloride) was collected and measured using UV spectrophotometer (Sytronics, Mumbai, India) at 287 nm using PBS pH 7.4 as blank. The concentration of entrapped moxifloxacin hydrochloride was determined spectrophotometerically after the lysis of the niosomal pellets with ethanol at 287nm, ethanol containing surfactant and cholesterol in the same ratio as in the niosomal formulations was used as a blank. 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 surfactant bilayers and/or aqueous compartments in the niosomes (Chengjiu *et al*. 1999). It was calculated using the following equation:

$$\text{Entrapment Efficiency (\%EE)} = \frac{\text{Amount of Moxifloxecin entrapped}}{\text{Total amount of Moxifloxacin incorporsted}} \times 100$$

2.3.3 Particle size and zeta potential

Niosomal vesicle sizes of the prepared formulae were determined by light scattering based on laser diffraction using Malvern Mastersizer (Malvern Instrument Ltd., Worcestershire, UK) (Junyaprasert *et al*., 2008). The measurements were performed using a 45mm focus objective and a beam length of 2.4mm. The samples diluted suitably by filtered water (0.5 micrometer filter-Himedia) were placed in the cuvettes and the procedure was carried out at 90° angle and temperature 25°C to determine the size of the particles in the range of 0.6nm to 3 microns. The zeta potential was determined using combination of laser Doppler velocimetry and phase analysis light scattering by Zetasizer Nano ZS-90 (Malvern Instruments Ltd.; UK). The diluted niosome dispersions were located in zeta meter cell for determination of electrophoretic mobility (Singh *et al*., 2011). All the experiments were performed in triplicate.

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2.4 *In vitro* drug release study

In vitro drug release studies of moxifloxacin from niosomes were carried out using membrane diffusion technique. *In vitro* diffusion cell was made using dialysis membrane as a semi permeable membrane. The diffusion cell consists of a test tube with both ends open. One end of the test tube was closed using presoaked dialysis membrane and the other end was open to introduce the niosomes formulation. An accurately measured amount of moxifloxacin niosomes formulation equivalent to 2 mg was suspended in 1 ml PBS (pH 7.4) and then transferred it to test tube, having a diameter of 2.5cm that was covered with a dialysis membrane (12000-14000 molecular weight cut off). The test tube was suspended in the dissolution flask of a USP dissolution apparatus containing 75 ml PBS pH 7.4. The temperature of the solution was maintained at 37±0.5 °C and the glass tube was allowed to rotate at a constant speed (50 rpm). Aliquots of the medium was withdrawn every hour and replaced with fresh PBS (pH 7.4). The samples were analyzed using UV/Visible spectrophotometer (AU-2701 Systronics, Mumbai, India) at 287 nm (Abdel-Mottaleb *et al.*, 2011)

2.5 Kinetic analysis of release data

To study the release kinetics, the data obtained from in vitro drug release studies were plotted in various kinetic models as follows (Srinivas *et al.*, 2010)

i.   Zero order: Cumulative % of drug released versus time ($Q_t = Q_0 - K_0 t$);
ii. First order: Log cumulative % of drug remaining versus time ($\ln Q = \ln Q_0 - K_1 t$);
iii. Higuchi: Cumulative % of drug released versus square root of time ($Q = K_H \sqrt{t}$);
iv. Hixson-Crowell cube root law: Cube root of initial concentration minus cube root of amount of drug released versus time ($Q_0^{1/3} - Q_t^{1/3} = K_{HC} \cdot t$)
v.   Korsmeyer–Peppas: Log cumulative % of drug released versus log time ($M_t/M_\infty = K_t^n$)

Where, Q_0, Q_t and K_t stand for the initial amount of drug, amount of drug release and kinetic release constant, respectively. M_t/M_∞ indicates the fractional drug release and "n" is the diffusional exponent which gives the mechanism of drug release dependent on the shape of matrix dosage form.

2.6 Transmission electron microscopy (TEM)

The morphology of the vesicles was examined by tramsmission electron microscopy (TEM). A drop of niosomal dispersion was diluted 10 times and was stratified onto a carbon-coated copper grid for 1 minute and excess was

removed by filter paper. A drop of 2% phosphotungstic acid solution was stratified to stain the vesicles; excess was removed by a tip of filter paper and left to air dry. The grid was observed by transmission electron microscopy (Hitachi, H-7500) and by using imaging viewer software the images were analyzed and captured (Muzzalupo *et al*., 2008).

2.7 Physical stability

Niosomal formulations were sealed into 10 ml vials and stored at refrigerator temperature (4°C) and at room temperature (25°C) for three months. The stability of the niosomal formulations were measured by the extent of to which encapsulated moxifloxacin was retained. This can be done by the determination of percent entrapment (%EE) as mentioned before, taking the initial %EE values as 100% (Shahiwala *et al*., 2002).

2.8 Ocular irritancy of moxifloxacin hydrochloride niosomes

A simple in vivo qualitative study was derived from modified Draize test for ocular irritation, without any scoring was performed to test the irritancy of Span type of surfactant used. The standard procedure involved the instillation of 0.1 ml of liquid (niosomal formulations) into the conjunctival sac of one eye and then gently holding the eye closed for one second. The untreated eye served as control. Both eyes were visually evaluated at 1, 6, 12, 24 48 and 72 hours after instillation.

3. RESULTS AND DISCUSSION

3.1 pH

pH is most important parameter especially in case of ophthalmic formulations. As the pH of the tear fluid is 7.4. Variation in the pH causes irritation in the eye. The pH of all the formulations was found in the range of 7.32 to 7.44. The detailed values are given in Table 2.

3.2 Entrapment efficiency

The entrapment efficiency was determined after separation of the un-entrapped drug by ultracentrifugation method. The entrapment efficiencies of all niosomal formulations are reported in Table 2. The encapsulation efficiency of moxifloxacin hydrochloride in niosomes is found to be highly dependent upon the ratio of cholesterol and surfactant at the same

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Table 2: Evaluation of Moxifloxacin Hydrochloride niosomes.

Formulation Code	Entrapment Efficiency (% ± SEM)	Particle Size (nm)	Zeta Potential (mV)	pH
MN 1	43.12±0.56	3651 ± 36	-15.1	7.34
MN 2	41.91±0.56	3297 ± 64	-14.8	7.38
MN 3	39.22±0.60	2305 ± 76	-14.9	7.41
MN 4	46.23±0.85	3792 ± 55	-22.3	7.38
MN 5	42.41±0.51	3402 ± 47	-21.6	7.39
MN 6	40.08±0.36	2669 ± 83	-22.5	7.40
MN 7	48.45±0.44	3961 ± 29	-30.3	7.32
MN 8	45.68±0.75	3580 ± 61	-29.0	7.40
MN 9	41.29±1.05	3091 ± 27	-29.83	7.41

time the concentration of DCP also plays a vital role in determining the entrapment efficiency. DCP, being a charged molecule is often used to prevent niosomes aggregation and increase the stability of niosomes dispersions. The results reviled that, the formulation containing maximum concentration of DCP increased entrapment of moxifloxacin in formulation. This may be due to fact that cholesterol in the presence of DCP was more efficiently able to stabilize the structure of niosomal membrane in molar ratio of (1:1) (span60- cholesterol). A direct proportionality was observed between the concentration of DCP and the entrapment efficiency (when the molar concentration of cholesterol and span 60 was constant) when used in concentrations between 2.5 to 7.5%. On the other hand the increasing surfactant concentration had a depreciating effect over the entrapment.
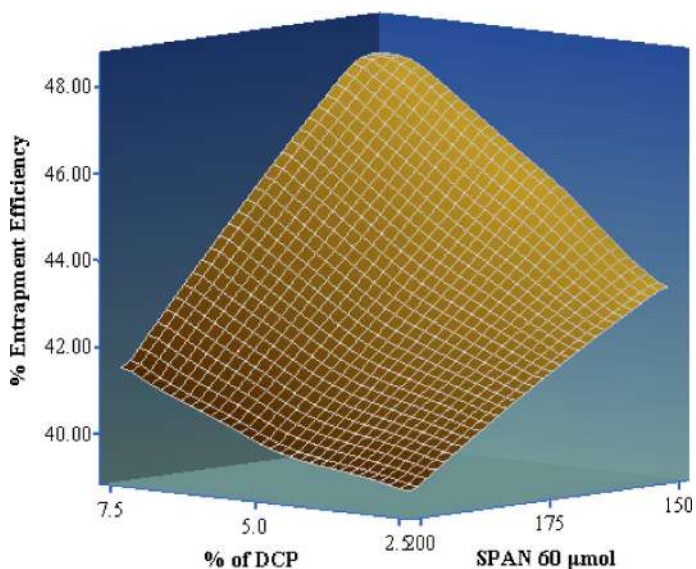
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Figure 1: Surface Response graph of Entrapment efficiency of Moxifloxacin Hydrochloride niosomes.

Surface Response graph of Entrapment efficiency of Moxifloxacin Hydrochloride niosomes is given in Figure 1. The small ratio or quantity of DCP i.e. 2.5% led to decrease in percentage entrapment efficiency of moxifloxacin. So it is clear that the higher quantity of DCP with in a composition of cholesterol and span 60 (1:1) molar ratio is most beneficial for the highest entrapment of moxifloxacin as per result i.e.48.45 ± 0.75% compared to the other formulation.

The Table 2 also showed that the lowest entrapment of moxifloxacin was obtained due to small amount of DCP i.e. 2.5% as compared to formulation containing MN8 i.e. 7.5%.

3.3 Particle size & Zeta potential

The particle size was determined from the graph obtained from dynamic light scattering method. The graph was obtained between particle diameter and percentage intensity. It was observed that increasing the concentration of DCP increases the particle size when used in the concentration range between 2.5 to 7.5%. This effect may be observed because the charge imparted by DCP causing the streaching of bilayer. On the other hand with increase in concentration of span 60 there was a marked decrease in the vesicle diameter. The highest

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Table 3: Release Kinetics Study of different niosomal formulations.

Formulation Code	Zero Order	First Order	Higuchi	Hixson Crowell	Korsmeyer Pappas Model	
	r^2	r^2	r^2	r^2	r^2	n
MN1	0 .8768	0.848	0.992	0.917	0.995	0.497
MN2	0.833	0.871	0.973	0.905	0.963	0.440
MN3	0.741	0.343	0.985	0.825	0.986	0.189
MN4	0.932	0.482	0.958	0.934	0.939	0.475
MN5	0.796	0.847	0.932	0.881	0.898	0.407
MN6	0.7014	0.847	0.956	0.808	0.957	0.303
MN7	0.895	0.823	0.957	0.879	0.946	0.500
MN8	0.925	0.895	0.9694	0.927	0.956	0.491
MN9	0.801	0.871	0.9646	0.892	0.934	0.378

vesicle diameter was obtained for formulation MN7 with a diameter of 3961 ± 29 nm shown in Figure 2 and formulation MN3 was characterized with the lowest particle size of 2305 ± 76nm. The surface response graph representing the effects of concentration of DCP and concentration of span 60 over the particle size of niosomes is given in Figure 2 and the particles size of different formulation is given in Table 2. Data in Table 2 & Figure 2 &3 show the Zeta potential of all the niosomes. Result showed that the zeta potential depends on the movement of particles (electrophoretic velocities). Result showed that niosomal formulation MN7 showed highest zeta potential -30.3 mV. The formulation MN2 showed lowest zeta potential. The value of zeta potential varied from -14.8 mV to -30.3mV. The presence of DCP incorporate negative charge in the niosomal suspension, this negative charge helps in depositing the

Table 4: Physical stability Study of optimized niosomes formulations.

Time (Days)	Refrigerator Conditions			Room Conditions		
	Entrapment Efficiency	% Drug Entrapped w.r.t initial Entrapment	Physical Appearance	Entrapment Efficiency	% Drug Entrapped w.r.t initial Entrapment	Physical Appearance
0	48.45%	100%	Opaque, Homogenous	48.45%	100%	Opaque, Homogenous
7	48.14 ±0.17%	99.37 ±0.35%	Opaque, Homogenous	47.79 ±0.29%	98.64± 0.60%	Opaque, Homogenous
15	47.12 ±0.22%	97.26 ±0.47%	Opaque, Homogenous	46.67 ±0.44%	96.33± 0.91%	Opaque, Homogenous
30	46.39 ±0.49%	95.74± 1.02%	Opaque, Homogenous	45.11 ±0.51	93.12± 1.06%	Opaque, Homogenous

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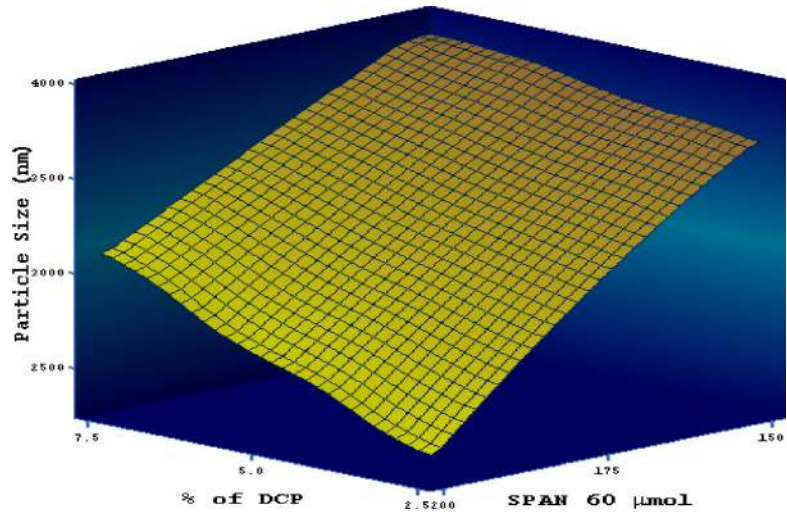
Figure 2: Surface Response graph representing the effect of different concentration of DCP and Span 60 on particle size.
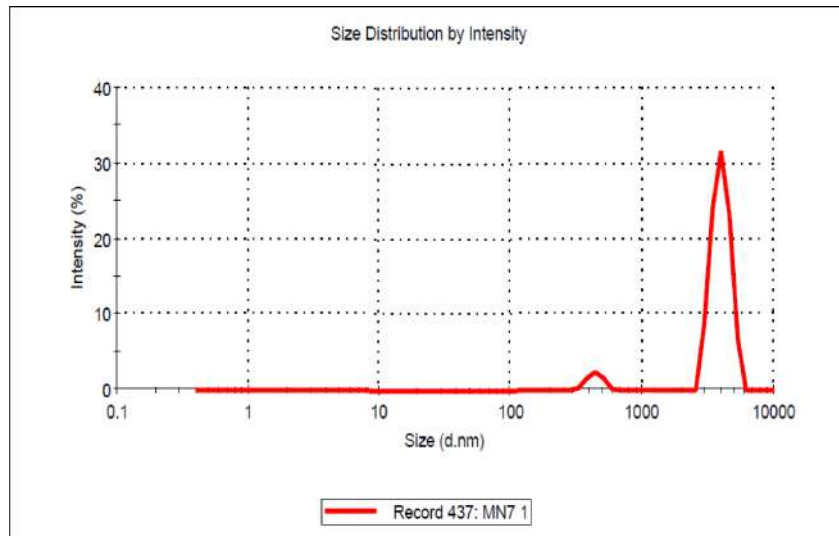

Figure 3: Size Distribution Curve of MN7 (3651 nm).

niosomal suspension over the cornea due to the electrostatic attraction between the positive charge of cornea and the negative charge of the niosomes.

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Table 5: Ocular irritation study of optimized niosomes formulations.

Time(hrs)	Rabbit 1	Rabbit 2	Rabbit 3	Average
1	2	1	2	1.67
6	2	1	1	1.33
12	1	0	1	0.67
24	1	0	0	0.33
48	0	0	0	0
72	0	0	0	0
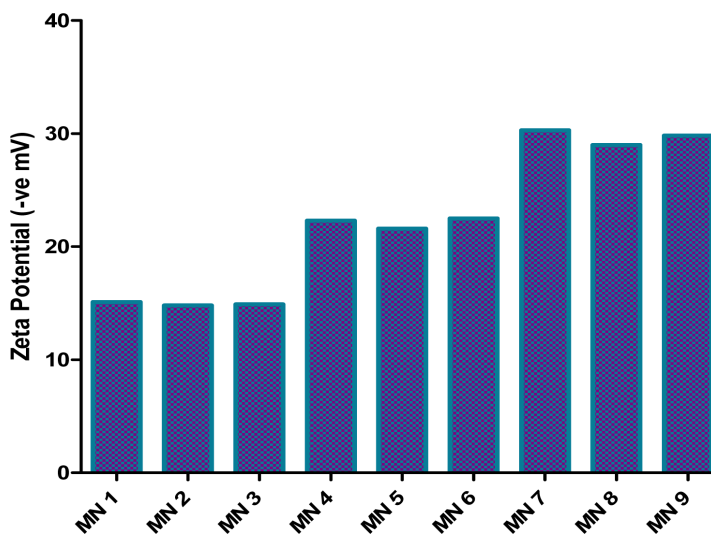
Figure 4: Zeta potential of Moxifloxacin Hydrochloride niosomes.

3.4 *In vitro* release studies

The *in vitro* drug release studies were carried out for all niosomal formulations using membrane diffusion technique (12000-14000 mol. Wt. cut off). The *in vitro* release of moxifloxacin from niosomal formulations by different molar ratio using different concentration of cholesterol, span 60 and DCP. The formulation containing maximum concentration of DCP i.e. 7.5% w/w provide retarded release, due to its stabilization action the niosomal formulations became less permeable. It is also concluded that the equal molar ratio of span 60 and cholesterol (1:1) and DCP concentration of 7.5% would provide stable
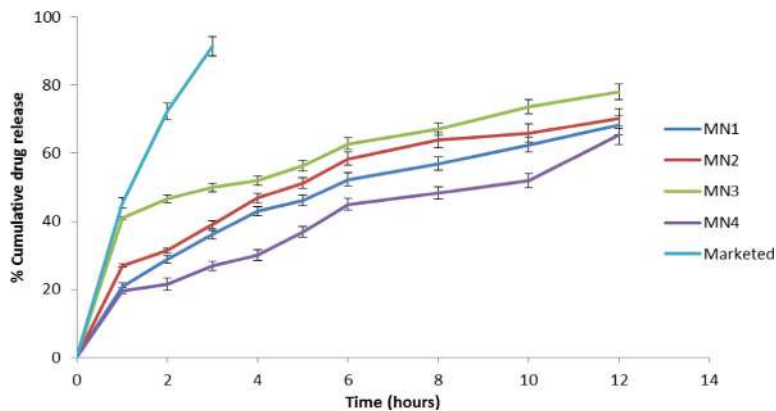
Figure 5: Cumulative release (%) profile of Moxifloxacin Hydrochloride niosomes (MM1-MN4).
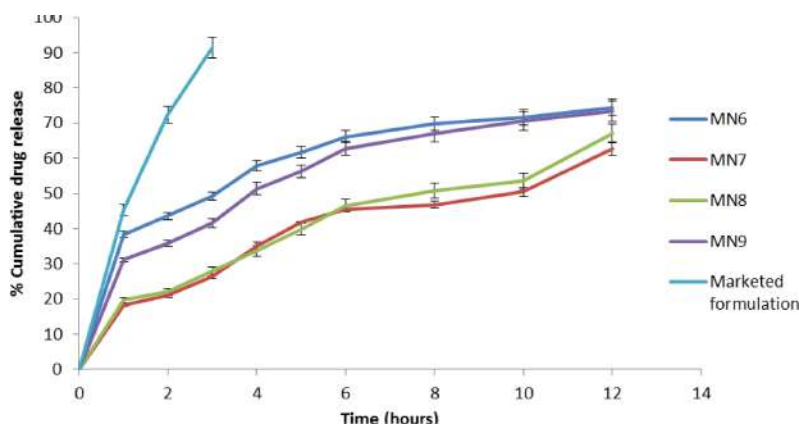

Figure 6: Cumulative release (%) profile of Moxifloxacin Hydrochloride niosomes (MN5-MN9).

niosomal formulation as compared to other prepared formulations. The result also showed that the combination of span 60 and cholesterol (1:1) proportions gave better sustained action of drug through niosomal formulations. The formulation MN3 (span 60: cholesterol) molar ratio produce faster release of drug i.e. 77.98% after 12 hours, concluded less sustained action. Niosomal formulations showed slower release rate than Moxifloxacin Hydrochloride marketed formulation. It also needed to note that in vitro release from niosomal formulation after 12 hours can be followed by decreasing order:MN3 > MN6 > MN9 > MN5 > MN2 > MN1 > MN8 > MN4 > MN7 (Figure 5 & 6).
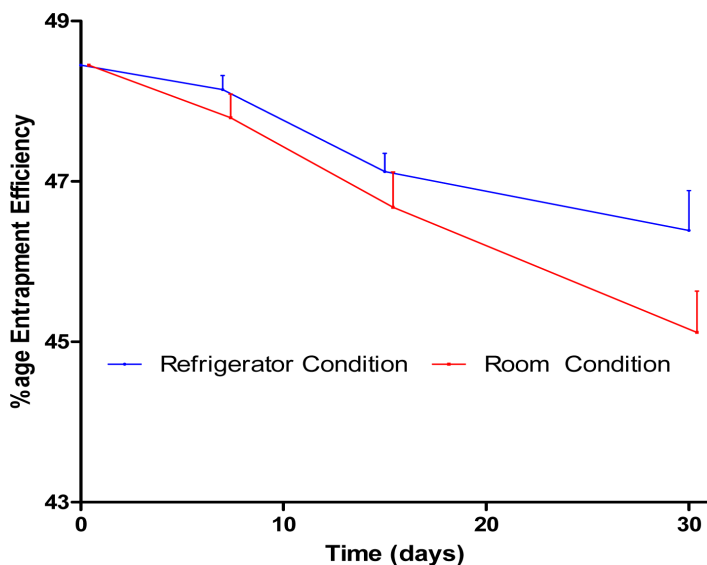
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Figure 7: Physical stability studies of Moxifloxacin Hydrochloride niosomes.

3.5 Release kinetics study

The drug release data obtained after *in vitro* release study was further analysed by various kinetic models plotted i.e. zero order, first order, Higuchi equation and Hixon Crowell cube root law. The regressed values of release profiles and the 'n' value of Korsmeyer Peppas model is given in table 3. All the formulations have shown to follow the higuchi kinetics as its coefficient of determinations (r^2) is much closer to one in most of the cases. The batches where the coefficient of determination has a value closer to 1 for Korsmeyer Peppas model have shown 'n' value either between 0.43 – 0.87 or their 'n' value is much lower than 0.43 so it can be visualized that Higuchi kinetics can be applied to the formulations with better accuracy and the formulations release the drug through non-fickian release kinetics.

3.6 Physical stability study

Physical stability study of optimized niosomes formulation MN7 were carried out to investigate the leaching of drug from niosomes by storing formulation under two different conditions i.e. in refrigerator (4-8 ^{0}C) and in room temperature (25^{0}C) for one month. The samples were withdrawn after 7th, 15th, 30th day, and analysed for entrapment efficiency. The results of stability studies are given in table 4 and in figure 7. The initial entrapment efficiency at the start

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of stability study was 48.45%. At cool temperature the formulation was stable over a period of 30 days and the percentage leaching of the drug in comparison to the initial entrapment was 4.26% in one month. The entrapment efficiency at refrigerator condition was found to be $46.39 \pm 0.49\%$ and at room condition was found to be $45.11 \pm 0.51\%$ at the end of one month stability period. At room temperature the percentage leaching of the drug in comparison of the initial entrapment was 6.88% in one month.

3.7 Transmission electron microscopy (TEM)

The prepared niosomal solution was a homogeneous dispersion and after maturation of 24 hours was studied under the microscope for morphological evaluation. The vesicles were uniform, spherical in shape with traces of aggregation. The formulations prepared with inclusion of DCP were found to be free from aggregation.

3.8 Ocular irritancy of niosomes

The results of the ocular irritation studies indicates that formulation MN7 having no redness, inflammation or increased tear production. Excellent ocular tolerance was noted. The results of the ocular irritation studies indicate that optimized formulation are non- irritant and well tolerated. The values of ocular irritancy are given in table 5. The ocular suspension showed mild irritation during the first six hours but irritation was reduced within 24 hours of application and no irritation was observed at 48 and 72 hours interval in any of the rabbits.

4. CONCLUSIONS

The present study conclusively supported niosomes to be an advantageous drug delivery system with high degree of entrapment and sustained release of the drug over extended period of time. It also suggests that the higher quantity of DCP with in a composition of cholesterol and span 60 (1:1) molar ratios is most beneficial for the highest entrapment of moxifloxacin. The careful control of all the above factors allow the production of a dosage form with sustained release capable of combating the side effects and also reducing the dosing frequency with the greater patient compliance.

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