Abstract

The design and development of a new ophthalmic drug delivery system with a view to enhance the efficacy of existing drugs is an ongoing process in the pharmaceutical research.

Such delivery systems include solid lipid nanoparticles (SLN), liposomes, niosomes, polymeric nanoparticles, microparticles and many others. These delivery systems are considered to be promising systems considering drug targeting to intraocular tissues, localizing drug at site of action and improving the transcorneal permeation of drugs.

Non-ionic surfactant based vesicles (niosomes) are formed from hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. They are usually used to incorporate hydrophilic drugs within their core; however hydrophobic drugs can be incorporated into niosomes lipophilic membrane.

Polymeric nanoparticles (PNP) are considered to be a promising drug delivery system to deliver medicinal agents into sites of action. Polymeric nanoparticles are solid colloidal particles with diameter ranging from 1 to 1000 nm. They have been recommended for delivering and targeting active agents owing to their particle size and long circulation in the blood. They consist of macromolecular materials and can serve as carriers in vaccines or drug carriers in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached.

Fluoroquinolones Antibiotics family has wide range of activity against serious infections caused by gram-negative organisms, including Pseudomonas species. The newer fluoroquinolones have a wider clinical use and a broader spectrum of antibacterial activity including gram-positive and gram-negative aerobic and anaerobic organisms.

Lomefloxacin hydrochloride is a fourth generation flouroquinolone that is widely used in ocular infections. However; it is subjected to many problems such as low residence time in the eye, washing out from eyes due to tear fluid turnover, poor

bioavailability due to poor transcorneal permeation. Subsequently; it is administered with high frequency which may lead to patient incompliance and thus decreasing the benefits obtained from such drug.

The above points make lomefloxacin HCl a good candidate for loading on drug delivery systems which can accumulate such a drug in the requited site of action leading to improvement of its therapeutic action.

The work in this thesis is divided into

- **Chapter I: Formulation and evaluation of Lomefloxacin HCl niosomes.**

This chapter includes the preparation and characterization of LF loaded niosomes. Also the in vitro release and transcorneal permeation studies of the prepared niosomes were performed.

In this chapter physicochemical properties of LF (spectrophotometric scanning and saturated solubility in simulated tear fluid (pH 5.5) and kribs ringer (pH 7.4)) were first evaluated.

Pre-formulation study was carried out to detect interaction between LF and niosomes components using Differential Scanning Colarimetry (DSC) and Fourier-transform Infrared spectroscopy (FT-IR).

Niosomes' preparation experiment was designed using 3^2 factorial design. Niosomes formulae were prepared using cholesterol, sorbitan monostearate (span 60), sorbitan monolaurate (span 20) and sorbitan monooleate (span 80) using thin film hydration method. The concentration of LF in each formula was 10 mg.

The prepared formulae were characterized with respect to encapsulation efficiency, particle size measurement by photo correlation spectroscopy (PCS), zeta potential, Transmission electron microscopy (TEM), scanning electron microscopy (SEM), in vitro transcorneal permeation study and in vitro release study.

In vitro permeation study through lipophilic membrane was performed using double open-sided tube with permeation area of 5 cm^2 and 50 ml receptor medium. The contents of the donor compartment (niosomes) and receptor compartment (krebs ringer

(ph 7.4)) were separated using bovine cornea. The receptor compartment was maintained at 37±0.5 ^{o}C while the donor compartment was left exposed to ambient temperature. The donor compartment's solution was stirred at 100 r.p.m by means of magnetic stirrer. Samples were withdrawn at specific time intervals and analyzed spectrophotometrically at 282 nm. The permeation parameters of different niosomes formulae along with plain drug were calculated.

Release experiments were carried out on niosomes formulae using reported method with modification. Certain volume of niosomes were placed in glass cylindrical tubes (7.5 cm in length and 2.5 cm in diameter) sealed at its lower end with dialysis membrane that were then attached to the paddles of USP dissolution tester (apparatus II). The temperature of the medium was set at 37±0.5^{o}C and speed of rotation was set at 50 r.p.m. samples were withdrawn at certain time intervals and diluted and the medium was compensated with equal volume of fresh medium. The linear regression analysis was applied to all in-vitro release results. The correlation coefficient (r^2) was determined in each case and accordingly, the orders of dissolution were determined.

Stability test was carried out on formula N2 by measuring encapsulation efficiency and particle size fresh and after a period of three months.

The collected data was statistically analyzed using One-way factorial ANOVA for comparing between different niosomes formulae and One-way ANOVA for comparing between niosomes and plain LF permeation data and in vitro release data.

From the obtained data in this chapter the following was concluded.

1-DSC and IR investigations revealed compatibility between LF and niosomes components.

2-LF was successfully embedded in niosomes core with encapsulation efficiency of LF reaching up to 78.10±1.23 % w/w, particle size that ranged from 109.4±1.0to 457.5± 1.5

nm with polydispesiy index ranging from 0.106±0.006 to 0.519±0.004 and zeta potential that ranged from -26.4±-0.1 to -43.6±-1.6 mV.

3- Formula **N6** gave the highest encapsulation efficiency of LF and the highest particle size while formula **N9** gave the lowest encapsulation efficiency of LF and lowest particle size

4- Formula **N3** gave the highest zeta potential, while formula **N9** gave the lowest zeta potential.

5-All the prepared niosomes formulae exhibited better transcorneal permeation parameters and slower release rate of LF compared to plain LF and formula **N9** had the best permeation parameters and release percentage of LF, while formula **N6** gave the lowest permeation parameters and release percentage of LF.

6- All prepared formulae had corneal hydration level that falls within the required range (76-80 %) so the prepared formulae were considered safe for ocular use.

7- Formula **N2** was selected to be used in further studies as it had reasonable entrapment efficiency (68.97±0.61 % w/w), particle size (242.8±3.0 nm), zeta potential (-34.8± 0.2 mV), drug percentage release after 8 hours (82.03±2.88) and good permeation parameters.

8-Formula **N2** was subjected to stability study for aperiod of three months. It was found to be stable for the stated storage period as there were non significant changes in the measured parameters.

Based on the data obtained in this chapter formula **N2** was chosen to be incorporated into three thermosensitive gel matrices for further study.

- **Chapter II: Formulation, evaluation and optimization of lomefloxacin HCl loaded polymeric nanoparticles.**

This chapter includes the preparation, characterization and optimization of LF loaded chitosan nanoparticles. Also the in vitro release and transcorneal permeation studies of the prepared nanoparticles were performed.

Pre-formulation study was carried out to detect interaction between LF and nanoparticles components using Differential Scanning Colarimetry (DSC) and Fourier-transform Infrared spectroscopy (FT-IR).

Modeling and optimization of nanoparticles was carried out using artificial neural networks (ANNs)-Genetic algorithm software package (INForm V3.6, Intelligensys Ltd., UK). Nanoparticles formulae were prepared using chitosan, sodium tripolyphosphate and sodium alginate at 3 concentrations of each using ionic gelation technique. The concentration of LF ranged from 10 to 15mg.

The prepared formulae were characterized with respect to encapsulation efficiency, particle size measurement by photo correlation spectroscopy (PCS), zeta potential, Transmission electron microscopy (TEM), in vitro transcorneal permeation study and in vitro release study.

In vitro permeation study through lipophilic membrane was performed using double open-sided tube with permeation area of 5 cm^2 and 50 ml receptor medium. The contents of the donor compartment (CS nanoparticles) and receptor compartment (krebs ringer (ph 7.4)) were separated using bovine cornea. The receptor compartment was maintained at 37±0.5 ^oC while the donor compartment was left exposed to ambient temperature. The donor compartment's solution was stirred at 100 r.p.m by means of magnetic stirrer. Samples were withdrawn at specific time intervals and analyzed spectrophotometrically at 282 nm. The permeation parameters of different nanoparticles formulae along with plain drug were calculated.

Release experiments were carried out on nanoparticles formulae using reported method with modification. Certain volume of LF loaded nanoparticles were placed in glass cylindrical tubes (7.5 cm in length and 2.5 cm in diameter) sealed at its lower end with dialysis membrane that were then attached to the paddles of USP dissolution tester (apparatus II). The temperature of the medium was set at 37±0.5^oC and speed of rotation was set at 50 r.p.m. samples were withdrawn at certain time intervals and diluted and the medium was

compensated with equal volume of fresh medium. The linear regression analysis was applied to all in-vitro release results. The correlation coefficient (r^2) was determined in each case and accordingly, the orders of dissolution were determined.

Stability test was carried out on the model optimized formula by measuring the encapsulation efficiency, particle size and zeta potential when fresh and after a storage period of three months.

The optimization model obtained solution for LF nanosuspension was composed of 11% LF, 0.1 % chitosan and 0.5 % sodium alginate as the crosslinker. The desirability of the obtained model approached 0.99 which represents how close the obtained model predictions are, to the desired values entered during optimization. The experimental evaluation of the optimized formula indicated similar properties where, the actual particle size was found to be 149.1 nm relative to the model predicted value of 176 nm. Also other insignificant differences between the actual and model predicted properties including ZP, PDI, % Rel-8hr, Q24, Lag time and Kp were also demonstrated.

From the obtained data in this chapter the following was concluded.

1-DSC and IR investigations revealed compatibility between LF and Cs nanoparticles components.

2-LF was successfully incorporated into CS nanoparticles with encapsulation efficiency of LF ranging between 80.03 ± 2.3 and 57.23 ± 1.99 % w/w, particle size that ranged from 57.03 ± 2 to 520 ± 3.5 nm, polydispersity index ranging from 0.016 ± 0.002 to 0.568 ± 0.012 and zeta potential (8.33 ± 1.6 to 40 ± 2 mV).

3- Formula **C21** gave the highest encapsulation efficiency of LF while formula **C1** gave the lowest encapsulation efficiency.

4- Formula **C7** gave the highest zeta potential, while formula **C12** gave the lowest zeta potential.

5- Formula **C1** had the lowest particle size and lowest PDI, while formula **C18** had the highest particle size and formula **C22** had the highest PDI.

6-All the prepared niosomes formulae exhibited better transcorneal permeation parameters and slower release rate of LF compared to plain LF and formula **C1** had the best permeation parameters and release percentage of LF, while formula **C18** gave the lowest permeation parameters and release percentage of LF.

7- Polymers concentrations (i.e. chitosan and crosslinkers) had major effect on nanoparticles characters and behavior as increasing polymers concentrations increased particle size and PDI. The medium concentrations of polymers gave the best entrapment efficiency. Increasing chitosan concentrations yielded an increase in zeta potential, on the other hand, increasing Na ALG and TPP concentrations resulted in a decrease in zeta potential values.

8- Increasing polymers concentrations showed a decrease in drug release rate and permeation parameters from CS nanoparticles.

9- All prepared formulae had corneal hydration level that falls within the required range (76-80%) so, the prepared formulae were considered safe for ocular use.

10- The experimental results of optimum formula deduced by the modeling software were highly similar to the model predicted results.

11- Optimized CS nanoparticles were found to be stable after storage period of three months concerning its entrapment efficiency, particle size and zeta potential values.

- **Chapter III: Formulation and evaluation of lomefloxacin HCl thermosensitive niosomal *in-situ* ocular gel**

In this chapter, pre-formulation studies to detect interaction between LF and the polymers used to prepare the thermosensitive base with three different mucoadhesives were performed using Differential Scanning Colarimetry (DSC) and Fourier-transform Infrared spectroscopy (FT-IR).

The selected formula of the LF loaded niosomes (**N2**) and LF solution were incorporated into themosensitive base (Poloxamers) containing three different mucoadhesive polymers namely chitosan (0.5% w/w), hydroxyl methyl propyl cellulose (HPMC) (1% w/w) and carpobole 941 (0.2%), all the prepared *in-situ* formulae had

0.3% (w/w) concentration of LF. The *in-situ* formulae were prepared using the cold method In case of poloxamers certain volume of distilled water, LF solution or LF niosomal suspensions were cooled down to 4°C. Poloxamers were then slowly added to the cold solutions with continuous stirring. In case of incorporating CS the medium was made slightly acidic by using acetic acid (0.5% v/v) and used as a solvent for the Poloxamers dispersion.

. In case of Cpb 941 containing formulae the medium was made a little bit basic by adding few drops of triethanolamine. Required amount of NaCl was added to all the formulation to make them isotonic. 0.01% (w/v) benzalkonium chloride added as preservative. The samples were then transferred into amber colored bottles and stored at 4°C over night to obtain clear solution prior to further analysis. Each in situ base was prepared in 3 formulae;

- In situ base containing no drug
- In situ base containing 100 % free drug
- In situ base containing 100 % LF loaded niosomes.

The prepared in situ formulae were characterized by visual inspection and measuring homogeneity percentage and spreadability.

The rheological properties of the prepared gel formulae were evaluated using a rotational Brookfield viscometer of cone and plate structure. About 0.5 g of the tested formula was applied to the plate and left until the temperature of the cone reached 25±1^{o}C. Measures were taken on a range of shearing rates from 20 to 400 (1/sec) corresponding to 10 to 200 r.p.m. Then, same procedure was repeated but at body temperature (37^{o}C) in order to detect the changes in rheological properties after sol-gel transformation.

The pH values determinations, gelation temperature values, isotonicity testing and mucoadhesion force measurements were done for all formulae.

In vitro permeation study was performed on the in situ formulae containing LF either in the plain form or encapsulated in niosomes in the same way that was followed in chapter I and II.

In vitro release study of In situ formulae was done as follows; a quantity of 1 gm of *in-situ* containing 0.3 % LF w/w was placed in glass cylindrical tube (7.5 cm in length and 2.5 cm in diameter) sealed at its lower end with dialysis membrane and suspended in 500mL of, STF pH 7.4 and placed in dissolution flask of USP dissolution apparatus and the temperature was kept at 37 ^{0}C and the tubes were allowed to stir at a velocity of 50 rpm. At predetermined time intervals. Samples of five mls were withdrawn and analyzed spectrophotometrically at λmax 282 nm. The medium was compensated with equal volume of fresh medium. The linear regression analysis was applied to all in-vitro release results. The correlation coefficient (r^2) was determined in each case and accordingly, the orders of dissolution were determined

The collected data was statistically analyzed using One-way ANOVA for comparison between different in situ formulae.

From the obtained data in this chapter the following could be concluded:

1- The results obtained from DSC and FT-IR analysis showed no interaction between LF and in situ bases.

2-All the prepared LF In situ formulae were elegant, transparent in case of plain LF gel and opaque in case of LF loaded niosomes. All formulae had good homogeneity and LF loaded niosomes in situ formulae had higher spreadability than plain *in-situ* and in situ containing LF solution, this increase in Spreadability was found to be significant using one way ANOVA at $p<0.05$.

3. The values of viscosity at room temperature were very low indicating that at room temperature the prepared formulae were in liquid state. Conversely at 35 ^{0}C, a dramatic thickening behavior was observed in the corresponding profiles of the formulations indicating temperature-induced gel structure formation of poloxamer.

4. *In-situ* formulae containing only Poloxamers had considerably high transition temperatures (higher than body temp.). Addition of mucoadhesives lowered the gelation temperature to be in the acceptable range. Niosomes containing formulae had high transition temperature compared to those containing free or no drug. F9 had the highest transition temperature, while, F6 exhibited the lowest transition temperature.

5. The mucoadhesive force measurements showed that the in situ formulae exhibited mucoadhesive force ranging from 1960±180 to 8820±181 dyne/ cm^2. Addition of mucoadhesive polymers resulted in a considerable increase in mucoadhesive force compared to plain Poloxamers.

6. The formulae containing LF either in solution or niosomal were found to be isotonic with blood when compared to marketed formula.

7. All the tested LF in situ formulae exhibited pseudoplastic flow with thixotropy. Formula F11 exhibited the highest area of hysteresis loop and formula F12 exhibited the highest Farrow's constant.

8. The permeation study showed that LF loaded niosomes in situ formula F11 had the best permeation parameters of LF, while formula F5 had the lowest permeation parameters. Also all formulations were considered quite safe for ocular use regarding the corneal hydration levels obtained.

9. The *in-vitro* drug release study of LF *in-situ* gel formulae showed that LF release from the *in-situ* gel formulae containing drug solution or niosomal LF was somewhat lower compared to LF release from drug solution and niosomal suspension of the drug. This could be due to increased viscosity of the medium due the used polymers.

10. Formula F6 had the highest release percentage of LF, while, formula F11 had the lowest release percent. All niosomes containing formulae showed lower release rate than those containing plain drug. Release of LF from formula containing plain LF in poloxamer and CS as mucoadhessive (F6) was higher than the formulae containing plain LF and HPMC or Cbp 971 (F7 and F8 respectively). Release of LF from Formula containing niosomal LF in poloxamers and Cbp 971 as mucoadhesive (F12) was higher

than release of LF from same matrices that contain niosomal LF and either CS or HPMC as mucoadhesives (F10 and F11 respectively). All gel formulae exhibited Higuchi diffusion type of release.

11- The One-way ANOVA test showed that there is significant difference between most of the prepared gel formulae considering release percentage of LF from the prepared LF in situ formulae.

- **Chapter IV: In-vivo performance and antibacterial activity of optimum LF loaded chitosan nanoparticles and LF loaded niosomal insitu gel**

The selected formulae from the previous study (F11 *in-situ* formula and optimum CS nanoparticles formula) were studied for their *in-vivo* performance and antimicrobial activity.

In this chapter ocular irritation test was first performed on tested formulae to make sure they are safe for ocular use. In this study; twelve New Zealand rabbits were divided into four groups each composed of three rabbits.

1. Group 1 received the optimized LF loaded CS nanosuspension in the right eye.

2. Group 2 received drug free optimized CS nanosuspension.

3. Group 3 received niosomal LF in situ gel

4. Group 4 received drug free niosomal in situ gel

The right eye was treated for one week twice daily and left eye taken as control in all animals. Eyes and eyelids were removed and kept in Davidson fixative solution for 24 hrs. They were cut in sections and stained with hematoxylin and eosin for histological examination.

In-vivo performance study of the selected formulae was designed as follows;

Group 1 received the marketed lomefloxacin HCl formulation (Orkacin 0.3 % w/v LF)

Group 2 received LF loaded CS nanosuspension (Optimized formula, 0.3 % w/v LF)

Group 3 received LF loaded niosomal in situ formula (Formula F11, 0.3 % w/v LF)

Rabbits were given 200 µL of the tested formulae every 15 min in both eyes for a period of one hour so that each animal received 1 mL (3 mg) in each eye. Drug concentration in different ocular tissues and fluids was determined using HPLC method.

Microbiological study was carried out on Optimized CS nanosuspension formula and F11 in situ gel formula, the antimicrobial activity of each was compared against LF solution. Minimum inhibitory concentration and minimum bactericidal concentrations values were determined by CLSI broth microdilution method.

Based on the results obtained in this chapter the following was concluded;

1- Ocular irritation test proved that the excipients used in formulating the tested formulae are non-irritant to eye tissues and are quite safe for ocular use.

2- The mean calibration curves obtained in different ocular tissues and fluids were highly linear in the concentration range 0.5 to 10 µg/ mL. The small intercepts indicated that the blank tissues extract and ocular fluids have negligible interference with drug.

3- The analysis of variance of the data of day to day reproducibility showed no detectable difference in the slopes of the three calibration curves at 5% significance level. The results thus confirmed excellent linearity of calibration curves and high reproducibility of the assay.

4- The results obtained showed that the mean drug concentrations in aqueous humor were 1.895 ± 0.2186, 9.892 ± 0.1529 and 10.360 ± 0.4547 µg/mL following administration of Orchacin ® eye drops, LF loaded CS nanoparticles and LF niosomal *in-situ* gel respectively.

5- The mean drug concentrations in vitreous humor were 1.661 ± 0.0227, 3.608 ± 0.1727 and 5.503±0.1572 µg/mL following administration of Orchacin ® eye drops, LF loaded CS nanoparticles and LF niosomal *in-situ* gel respectively.

6- Corneal drug concentrations were found to be 3.750 ± 0.0367, 8.943 ± 0.3480 and 10.312 ± 0.1153 µg/mL following administration of Orchacin ® eye drops, LF loaded CS nanoparticles and LF niosomal *in-situ* gel respectively.

7- Conjunctival drug concentrations were found to be 2.198 ± 0.0126, 3.763 ± 0.0315 and 6.756 ± 0.0516 µg/mL following administration of Orchacin ® eye drops, LF loaded CS nanoparticles and LF niosomal *in-situ* gel, respectively.

8- The difference between drug concentrations in different tissues and eye fluids were found to be significant at 5% level of significance.

9- The tested formulae showed statistically significant higher antibacterial activity against Gm –ve and Gm +ve bacteria compared to free LF solution.

The results obtained through out the work reveal that incorporating ocular antibiotics into niosomes and polymeric nanoparticles increased drug residence time in eye and allowed deep penetration through out ocular tissues to deliver drug into posterior eye segments and help treating deep ocular infections. The results also proved that such drug delivery systems can enhance the antimicrobial activity of antibiotics and hence maximize the benefits obtained from such drugs.