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Formulation and evaluation of non-ionic surafactant vesicles (niosomes) for ocular delivery of ofloxacin

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Abstract

The aim of the present investigation was to formulate and evaluate Niosomes, a synthetic microscopic vesicles consisting of an aqueous concentration enclosed in a bilayer consisting of cholesterol and one or more nonionic surfactants to improve the low corneal penetration and bioavailability characteristic shown by conventional ophthalmic vehicles. Ofloxacin was selected as a suitable drug for the present study because it is a potent second-generation fluoroquinolone active against a broad range of gram positive and gram-negative aerobic and anaerobic bacteria. In the present investigation nine formulations of Niosomal drug delivery system of ofloxacin with non ionic surfactant, span 60, in various proportions were prepared and evaluated for Morphological characterization, Encapsulation efficiency, *In-vitro* drug release study, Drug release kinetic data analysis, Stability study, Test for significance, Zeta potential analysis, Ocular irritation test, Estimation of minimum inhibitory concentration, *In- vivo* study. Niosomes formed from span 60 and cholesterol in the ratio 200:100 (in µmol) is a promising approach to improve the bioavailability of ofloxacin even for an extended period of time which showed good physicochemical properties, good stability and controlled drug release pattern, thereby improving the bioavailability of the drug.

Keywords: Niosome, Ofloxacin, In-Vitro Release, Bioavailability.

Introduction

Ofloxacin 0.3% ophthalmic solution indicated for treatment of postoperative inflammation in patients suffering from acute and sub-acute conjunctivitis, bacterial corneal ulcer , bacterial keratitis or kerato conjuctivities Ofloxacin is a broad-spectrum antibacterial agent with activities against gram-negative bacteria (*E. coli, Klebsiela pneumoniae, Serratia* species, *Proteus* species, *Pseudomonas aerogenosa* and *H. influenzae*) and gram-positive bacteria (*Staphylococcus species, Streptococcus enterococci*). The recommended dosage regimen for the treatment of bacterial conjunctivitis is: days 1 and 2 - Instill one to two drops every two to four hours in the affected eye(s).days 3 through 7 - Instill one to two drops four times daily. [1-2]

The rate of drug penetration depends not only on the physicochemical properties of the drug itself, such as its solubility and particle size, in case of suspensions but also on those of its vehicle. Vesicular systems have gained considerable attention for drug delivery as it has property of crossing cell membrane. Vesicles, therefore, can be viewed as drug carriers and as such they change the rate and extent of absorption as well as the disposition of the drug. Vesicular drug delivery systems used in ophthalmic delivery broadly include liposomes and niosomes that cationic liposome prepared by reverse phase evaporation can increase the corneal contact time, enhance the corneal permeability of ofloxacin and thus improve ocular bioavailability. Niosomes were primarily investigated as a system which increases the time of release and decreases the systemic absorption.[3]

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In the present study, niosomal suspension ofloxacin prepared with various concentration of cholesterol and surfactant (Span 60). Span 60, being widely used and established surfactant in terms of its capability of providing high entrapment efficiency and prolong release, is used in the present study (A. S. Guinedi et al,). The prepared niosomes were characterized for entrapment efficiency and in vitro release. The stability and in vivo performance of the formulation were also studied.

Material and methods

Ofloxacin was obtained as gift from Microlabs, Bangalore (India). Cholesterol and Span 60 were purchased from Loba chem. (India). Chloroform, Glacial acetic acid Acetonitrile, Sodium chloride, Potassium dihydrogen phosphate, Sodium hydroxide and Di. Sodium hydrogen phosphate was purchase from S. D. Fine (India). Xylocaine 4%, Astra Zenica (India). All chemicals used in experiments were of analytical grade. Dialysis membrane 50 was purchased from Hi media.

Preformulation study[1]

The FT-IR spectrum of pure drug was analyzed to check the purity of the drug using Shimadzu Fourier Transform Spectrophotometer by KBr pellet method. The IR absorption spectra of the pure drug were taken in the range of 400-4000 cm^{-1}.

Preparation of niosomes[1-2]

The ofloxacin niosomes were prepared by lipid film hydration technique. Drug (ofloxacin), non ionic surfactant and cholesterol were weighed (surfactant: cholesterol in µmol) and dissolved in chloroform / Glacial acetic acid (2:1) in a 100 ml round bottom flask. A thin lipid film formed under reduced pressure in a rotary flash evaporator. The film then hydrated by 10 ml of PBS 7.4 at room temperature by gentle shaking. The suspension was then sonicated for 3 x 30 seconds to form small unilamellar vesicles.

Microscopy[2]

The vesicle formation by the particular procedure was confirmed by optical microscopy in 400x resolution. The niosome suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film of niosome suspension observed for the formation of vesicles.

Entrapment efficiency[3]

Niosome entrapped ofloxacin was estimated by ultracentrifugation method. The entrapment efficiency of niosomes was determined by ultracentrifuging the niosomal dispersions at 40,000g for 30min.the clear supernatant was analyzed for ofloxacin Spectrophotometrically and gave the amount of unentrapped drug. Amount of entrapped drug was obtained by subtracting amount of unentrapped drug from the total drug incorporated.

$$\text{Percent Entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \times 100$$

***In-vitro* drug release study**[3]

In vitro release pattern of niosomal suspension was carried out in dialysis bag method. 1.5 mg equivalent of 0.3 % of niosomal suspension was taken in dialysis bag (Hi media) and the bag was placed in a beaker containing 250 ml simulated tear fluid (pH7.4 phosphate buffer). The beaker was placed over magnetic stirrer and the temperature was maintained at 37 ± 1^0C. 5 ml samples were withdrawn periodically and were replaced by fresh buffer. The sink condition was maintained throughout the experiment. The withdrawn samples were analyzed for drug content using U.V. spectrophotometer at 294 nm keeping phosphate buffer pH 7.4 as blank.

Physical stability[4-5]

Physical stability carried out according to the method specified. Best three of the optimized ofloxacin niosomal suspension sealed in glass vials and stored in refrigerated temperature ($2\text{-}8^0$C) for a period of 3 months. Samples from each batch were withdrawn after the definite time intervals and the residual amount of drug in the vesicles was determined. Stability data of three formulations were further analyzed for significant difference by paired t-test.

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Zeta potential analysis[3]
Zeta potential was analyzed to measure the stability of niosome by studying its colloidal property. The study was conducted using zeta potential probe (model DT-300).

Ocular irritance test[6-7]
The potential ocular irritation and/ or damaging effects of the niosomes under test were evaluated by observing them for any redness, inflammation (or) increased tear production. Formulation was tested on six rabbits by dispensing niosomes in the cul-de-sac of the left eye.

Estimation of minimum inhibitory concentration (MIC) of ofloxacin
MIC is the lowest concentration of drug that prevents growth of a particular pathogen. Test can show which agents are most effective against a pathogen and gives an estimate of the proper therapeutic dose. The antibiotic ofloxacin in various concentration range 1-10 µg/ml was added in a series of nutrient broth tubes inoculated with standard test organism (Staphylococcus aureus- Gram positive bacteria). The lowest concentration of the antibiotic resulting in no growth (indicated by no turbidity) after incubation for 24 hr is called as the MIC of ofloxacin for Staphylococcus aureus.

***In Vivo* Study**[8-10]
Male rabbits (*Orytolagus cuniculus*), 10-12 weeks old, weighing 2.5-3 kg were used in the present study. They were housed individually with husk bedding and fed with standard laboratory diet and water as much as required.. The temperature was maintained at 28±2°C. The study protocol was approved by Institutional Animal Ethical Committee for the use of animals in the research. Two healthy rabbits were used for the study. For the measurement of ofloxacin transcorneal penetration into the aqueous humor, Three drops of 0.3% niosomal suspension of ofloxacin was instilled in the lower cul-de-sac of each eye. The upper eyelids were gently held closed for 10 seconds to maximize the corneal contact. At the 5th and 10th hour after instillation of niosomes, eyes were anesthetized using 4% Xylocaine solutions topically and 50 µl aqueous humor was sampled from eyes by introducing a 26 gauge needle between the junction of sclera and cornea. After extraction the eyes were treated with ciprofloxacin eye drops for the prevention of infection. The aqueous humor samples were immediately frozen and stored at -18 °C. For analysis, each sample was mixed with an equal volume of acetonitrile, then it was centrifuged at 13000 rpm for 15 minutes and 20 µl of the supernatant was analysed for the presence of ofloxacin by HPLC- U.V detector, by comparing with the HPLC peak of standard solution of ofloxacin (100 µg/ml). There exists the possibility of drug binding with the aqueous humor proteins resulting in a reduction in the free drug concentration

Chromatographic details

- Mobile Phase : methanol:acetonitrile:citric acid 0.4 M (3:1:10)
- Flow Rate : 1.0 ml/min
- Column : C_{18} universal column
- Detector : UV-detector at 294 nm
- Retention time : 6.8

Results and Conclusion

Morphological characterization
The prepared vesicles were studied under 400 x magnifications to observe the formation of vesicles. Some unevenness of vesicles that observed under the study may be due to drying process under normal environment condition. The photomicrograph of niosomes is shown in the Fig.1

Entrapment efficiency
Entrapment efficiency was studied for all the 9 formulations to find the best in terms of entrapment efficiency. The entrapment efficiency was found to be higher with the formulation no. F_5 (80.10%), which may have an optimum cholesterol surfactant ratio to provide a high entrapment of ofloxacin. The niosomal formulations having high surfactant concentration (F_3, F_4 and F_5) have the higher entrapment efficiency which might be due to the high fluidity of the vesicles. Very low cholesterol content (F_6) was also found to cause low entrapment efficiency

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(57.43%), which might be because of leakage of the vesicles. It was also observed that very high cholesterol content (F_7) had a lowering effect on drug entrapment to the vesicles (45.23%). This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment. Entrapment efficiency showed by various formulations are specified in Table 1

***In-vitro* release profile**

The release study was conducted for all the nine formulations. Most of the formulations were found to have a linear release and the formulations were found to provide approximately 65% release within a period of 10 hours. The formulations which have high cholesterol ratio (F_7, F_8) were found to sustain the drug release. Cholesterol, which has a property to abolish the gel to liquid transition of niosomes, this found to prevent the leakage of drug from the niosomal formulation. The slower release of drug from multilamellar vesicles may be attributed to the fact that multilamellar vesicles consist of several concentric sphere of bilayer separated by aqueous compartment. The above specified three best formulations F_3, F_4, and F_5, were found to give a cumulative release of 68.263%, 69.250%, and 73.287% respectively over a period of 10 hrs, the higher release from the formulation F_5 may be because of its low cholesterol content. Formulations F_1, F_7 and F_8 having the highest cholesterol content showed the lowest release over 10 hours, they provide a release of 59.12%, 55.62% and 56.12% respectively .comparative release study is shown in Graph 2 and 3

Physical stability

Physical stability was carried out to investigate the leaching out of the ofloxacin from niosomes at refrigerated temperature, as shown in the graph no: 31. The percent of ofloxacin retained in the span 60 vesicle after a period of three months were 80.13% 76.54% and 73.98 % respectively for formulations F_5 (200:100), F_4 (200:125) and F_3(200:150). Also the results indicate that more than 80% of ofloxacin was retained in the niosomal formulation for a period of 60 days. From this it can be concluded that vesicles are stable enough to store under refrigeration temperature with least leakage as shown in Graph 1.

Zeta potential analysis

The formulation F_5 which was subjected to zeta potential analysis had a zeta value of +31mv, which is a measure of net charge of niosomes. This higher charge on the surface of vesicle produce a repulsive force between the vesicles which made them stable, devoid of agglomeration and faster settling, providing an evenly distributed suspension. From this it can be concluded that formulation F_5 provides much stable niosomal suspension.

Ocular irritation test

The rabbits subjected to ocular irritation test did not show any signs of irritation, inflammation or abnormal discharge. Niosomes (F_5) appeared to be devoid of any irritant effect on cornea, iris, and conjunctiva up to 24 h after application, which probably suggests its suitability for ophthalmic drug delivery. However, the animal behavior was slightly agitated from the normal animals but the intake of food and water was normal.

Estimation of minimum inhibitory concentration (MIC) of ofloxacin niosome

The minimum inhibitory concentration of ofloxacin was found to be 4.375µg/ml. This is the lowest concentration at which there was no growth of bacteria S.aureus as indicated by no turbidity. But the concentration below 4.375µg/ml there was growth of the bacteria as indicated by turbidity

***In-vivo* study**

The prepared niosomes of ofloxacin (F_5) studied for its prolonged release *in vivo* in the animal model Rabbit. *In vivo* study conducted to investigate the ocular availability of drug for a prolonged action after a single dose. The study carried out by comparing the retention time of the standard drug solution to that of the aqueous humors extracted sample. The retention time obtained for the standard was 6.98 minutes, for the samples at 5^{th} and 10^{th} hour were 6.92 and 6.85 minutes respectively. This matching retention time of three injections to HPLC showed the presence of drug in the aqueous humor sample even after 5^{th} and 10^{th} hour of administration. In other words drug was available in detectable quantities even after 10^{th} hour of administration, where as the literature says, concentration of ofloxacin in aqueous humor will be in undetectable quantity after 5^{th} hr of administration as conventional ocular drops. This may be because of possible retention of drug in the aqueous humor due to high corneal contact time and permeability provided by the vesicular system.

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Fig. 1: Photomicrograph of niosome in a dry glass slide

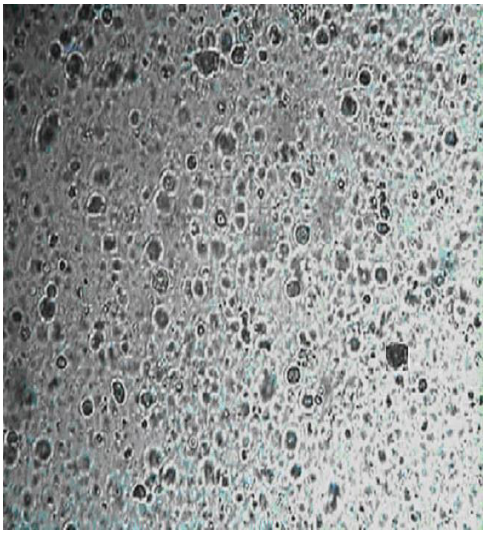
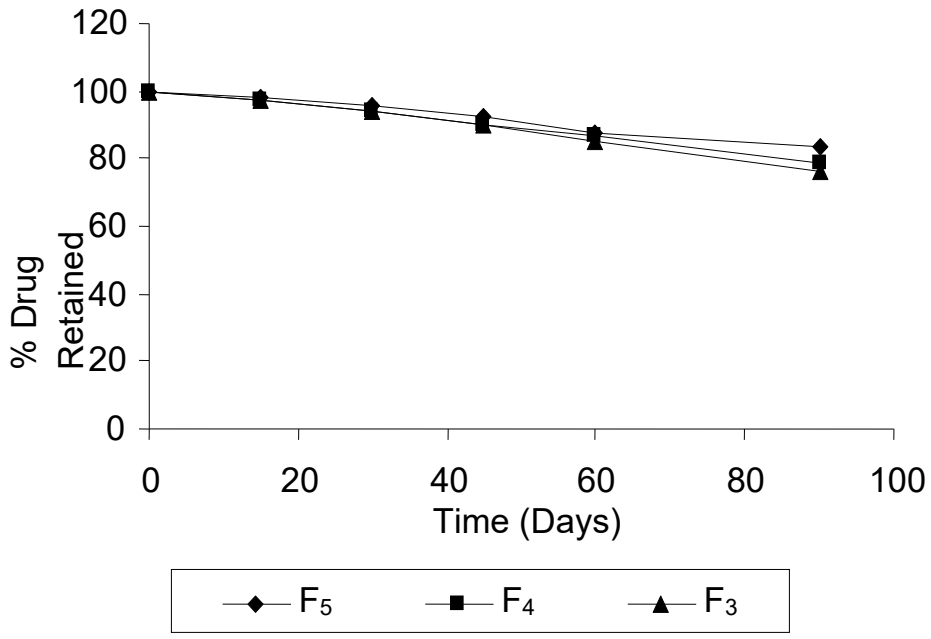


Table 1: Entrapment efficiency of various formulations

S.No.	Formulation no.	Molar ratio (Span60 : Cholesterol)	Entrapment efficiency %
1	F_1	200:200	61.34
2	F_2	200:175	63.12
3	F_3	200:150	67.48
4	F_4	200:125	71.24
5	F_5	200:100	80.10
6	F_6	200: 75	57.43
7	F_7	150:200	45.23
8	F_8	150:150	68.72
9	F_9	150:125	58.23

Graph 1: Percentage of ofloxacin retained in the niosome formulations after storage at refrigeration temperature



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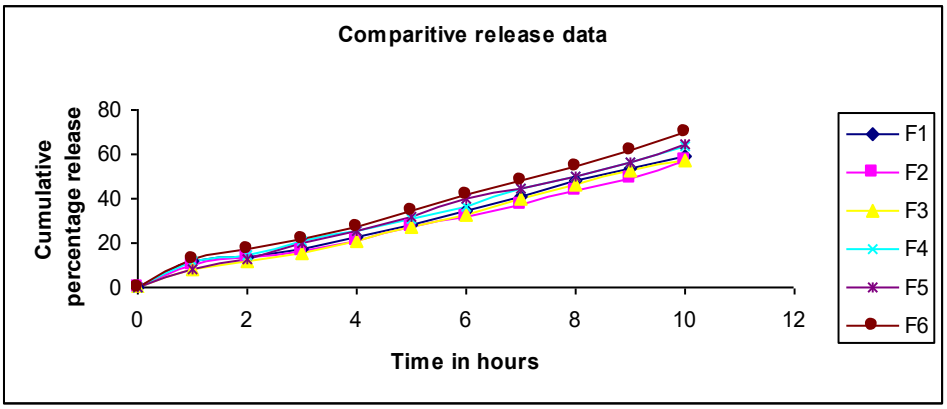
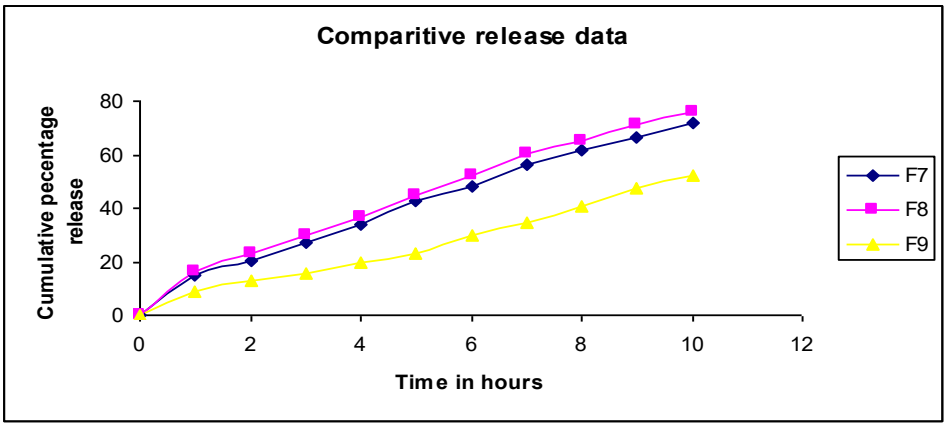
Graph 2: Comparative release data F1 to F6



Graph 3: Comparative release data F1 to F6



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