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Preparation and characterization of ofloxacin non-ionic surfactant vesicles for ophthalmic use

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ABSTRACT

Ofloxacin is a fluorinated 4-quinolone antibiotic which is used in the treatment of ophthalmic ailments like infections, inflammations, conjunctivitis, blepharitis, iritis, corneal ulcer etc. Commercially, ofloxacin eye drop solution is available and it is quite easy for the administration. However, the product has drawback of poor bioavailability due to several factors such as tear production, non-productive absorption, transient residence time, and impermeability of corneal epithelium. In order to improve the bioavailability, residence time and longer duration of action, an attempt was made to formulate the non ionic surfactant vesicle and characterized. In the present study, the nonionic surfactant vesicles were prepared by lipid film hydration method using span 60 and cholesterol with various molar ratios and characterized for entrapment efficiency, in-vitro drug release, surface charge, rheological character, physical stability, minimum inhibitory concentration, in-vivo drug release and ocular irritation were conducted. The span 60: cholesterol in molar ratio of 100:60 showed higher entrapment of drug and released 73.77 % at 10th h and the availability of drug in the aqueous humor was 4.373µg/ml (Cmax), confirmed by HPLC method. The histopathology study also confirmed the safe use of niosomes. Study may be concluded that the non-ionic surfactant vesicles formulated with span 60 and cholesterol in a molar ratio of 100:60 showed potential approach to improve the ocular bioavailability of ofloxacin for the prolonged period of time.

Keywords: Niosomes, Ocular delivery, Ofloxacin,

INTRODUCTION

Most of the drugs meant for ocular treatment are intended to the tissues around the ocular cavity. Though there are several types of ophthalmic dosage forms are available for the ophthalmic ailments, topical administration of conventional eye drop solution is widely accepted or preferred over systemic administration so as to avoid systemic toxicity, rapid onset of action and decreasing the required dose[1]. This conventional dosage forms account for 90% of the available ophthalmic formulations[2].

Infections, inflammations, conjunctivitis, blepharitis, iritis, corneal ulcer etc. are quite common in ocular infections which are primarily treated with ofloxacin by the ophthalmologists. Ofloxacin is a fluorinated 4-quinolone antibiotic and is active against a wide spectrum of gram positive and gram negative organisms including various species of staphylococcus, streptococcus, and pseudomonas[3]. It inhibits the enzyme bacterial DNA gyrase, which nicks double stranded DNA, introduces negative supercoils and then reseals the nicked ends. This is necessary to prevent excessive positive supercoiling of the strands when they separate to permit replication or transcription. The bactericidal action probably results from digestion of DNA by exonucleases whose production is signaled by the damaged DNA[4].

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The application of ofloxacin eye drop requires strict discipline from the patient or care provider. Moreover, frequent instillation of the concentrated solutions may damage the ocular surface[5]. However, ocular bioavailability after topical ocular eye drop administration is less than 5% and often less than 1%[6]. Poor bioavailability of drugs from ocular dosage form is mainly due to the tear production, non-productive absorption, transient residence time, and impermeability of corneal epithelium[1].

In order to enhance the bioavailability of ofloxacin by topical route and to improve the corneal permeability of the drug, the niosomes of ofloxacin were prepared[7]. Niosomes have been reported as a possible approach to improve the low corneal penetration and bioavailability characteristics shown by conventional ophthalmic vehicles[8]. Nonionic surfactant based vesicles are formed from the self assembly of nonionic amphiphiles in aqueous media resulting in closed bilayer structures[9] which can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in vesicular membrane[10].

Niosomes used in ophthalmics drug delivery system help in providing prolonged and controlled action at the corneal surface and preventing the metabolism of the drug by enzymes present at the tear/ corneal surface[1]. Drug enclosed in the non ionic surfactant vesicles allows for an improved partitioning and transport through the cornea. In addition, vesicles offer a promising avenue to fulfill the need for an ophthalmic drug delivery system that has the convenience of a drop, but will localize and maintain drug activity at its site of action[11]. In the present study, it was aimed to prepare non-ionic

surfactant vesicles for ocular use containing ofloxacin with better bioavailability and longer duration of action.

MATERIALS AND METHODS

Materials

Ofloxacin was obtained from Microlabs, Bangalore, India, as a gift sample. Sorbitan monostearate (span 60), cholesterol and chloroform were purchased from Loba chem. Pvt ltd, Mumbai. Methanol, Potassium di-hydrogen phosphate and Disodium hydrogen phosphate were purchased from S.D fine chem. Ltd, Mumbai, India. Sodium hydroxide from Nice chem. Ltd, Mumbai. Xylocaine from Astra zenica Ltd, India. Distilled water from Leo scientific, Erode,T.N, India and HPLC water was purchased from Qualigens, India.

Methods

Preparation of Ofloxacin Niosomes

The niosomes were prepared by lipid film hydration method[12, 13]. Accurately weighed quantity of ofloxacin and surfactant, cholesterol in different molar ratios such as 100:30, 100:40, 100:50, 100:60, 100:70 and 100:100 were dissolved in chloroform / methanol (2:1, v / v) in a 100 ml round bottom flask. A thin lipid film was formed under reduced pressure in a rotary flash evaporator at 60º C. The film was then hydrated, after the removal of last trace of organic solvent, by 10ml of phosphate buffer saline pH 7.4 at 60º C for one hour. The prepared niosomal suspensions was mechanically shaken for one hour using horizontal mechanical shaker at 60rpm and 40ºC leading to the formation of multilamellar niosomes. The niosomal suspension was left to mature overnight at 4ºC.

Entrapment Efficiency[14]

Ofloxacin niosomal suspension prepared by lipid film hydration method was subjected for the determination of Entrapment efficiency by dialysis method. The prepared samples were taken into the dialysis bags and the free ofloxacin dialyzed for 24h into 100ml of phosphate buffer saline pH 7.4. The absorbance of the dialysate was measured against phosphate buffer saline pH 7.4 at 294nm and the absorbance of the corresponding blank niosome was measured under the same condition. Amount of entrapped drug was obtained by subtracting amount of unentrapped drug from the total drug incorporated.

$$\text{Percentage entrapment} = \frac{\text{Entrapped drug (mg)}}{\text{Total drug added (mg)}} \times 100$$

In-Vitro Release of Ofloxacin from Niosomes[8]

The dialysis bag method was carried out to study the *in-vitro* drug release pattern. 2mg equivalent of 0.1% of niosome disperesion was taken in dialysis bag and the bag was placed in a beaker containing 100ml simulated tear fluid, pH 7.4 phosphate buffer saline. The beaker was placed over magnetic stirrer and the temperature was maintained at 37±1º C. Samples were collected at predetermined time and replenished immediately with the same volume of fresh simulated fluid. The sink condition was maintained through out the experiment. The withdrawn samples were analyzed for the drug content after suitable dilution using UV spectrophotometer at 294nm, keeping phosphate buffer pH 7.4 as blank.

Zeta Potential Analysis[15]

Zeta potential analysis was used to measure the stability of niosome by studying its colloidal property. The presence of surface charge in vesicular dispersion is critical. Aggregation is attributed to the shielding of the vesicle surface charge by ions in solution and there by reducing the electrostatic repulsion. Vesicle surface charge can be estimated by measurement of particle electrophoretic mobility and is expressed as the zeta potential. The study was conducted using zeta potential probe (model DT 300)[13].

Rheological Character[16]

The viscosity of the prepared niosome suspension was studied using Brookfield viscometer LVDV-E. 6.7ml of niosomal suspension was accurately measured and introduced into the SC4 series small sample chamber -13R. The spindle 18 at 100 RPM and shear rate 1.32 N (Sec^{-1}) were used.

Physical Stability[8]

The leaching of drug from niosomal suspension during storage was studied with surfactant, cholesterol in molar ratio of 100:60. The sample was sealed in 20ml glass vials and stored in refrigerated temperature (2-8^{0}C) for a period of 60 days. At definite time intervals, samples from each batch were withdrawn and the residual amount of drug in the vesicles was determined by dialysis method.

Estimation of Minimum Inhibitory Concentration (MIC) of Ofloxacin[17,26]

The various concentration of ofloxacin between the ranges of 1-10 µg/ml was introduced into the series of nutrient broth tubes and inoculated with standard test organism (Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumonia and Pseudomonas aeroginosa) to find out the MIC of drug in terms of the lowest concentration of drug that prevents growth of a particular pathogen. The lowest concentration of the antibiotic resulting in no growth indicated by no turbidity after incubation for 24h was considered as MIC of ofloxacin for the above said organisms.

In-Vivo Study[3,7]

In the present study, six male albino rabbits 10-12 weeks old and weighing 2.5-3.5 kg were used. The rabbits were provided with food and water ad libitum in a temperature controlled room (18-24ºC). The study protocol was approved by Institutional Animal Ethical Committee (688/2/C/CPCSEA - NCP/IAEC/02/2006-2007) for the use of animals in the research work. The surfactant, cholesterol in molar ratio of 100:60 ofloxacin was instilled in the lower cul-de-sac of right eye. The upper eyelids were gently held closed for 10sec to maximize the corneal contact. Using 28 gauge needle, the aqueous humor samples were collected between the junction of sclera and cornea of right eye at various time intervals between 40 - 360mins of post dose after anesthetizing the eyes using 4% xylocaine solutions topically, whereas left eye was treated as control (Oflox®,Protec). The eyes were treated with ciprofloxacin eye drops for the prevention of infection after the extraction. Sampled aqueous humor was then mixed with 100µl of ethyl acetate and kept in the refrigerator for one hour. The mixture was then centrifuged at 3000rpm for 20mins and the supernatant obtained was analyzed for the presence of ofloxacin by HPLC.

Assessment of Ocular Irritancy of Niosomes[11]

Six healthy rabbits weighing 2.5-3kg were selected for the ocular irritancy test. The potential ocular irritancy and/ or damaging effects of the formulations under test were evaluated by observing them for any redness, inflammation, or increased tear production. The

right eye of each rabbits received niosomal suspension of span 60 and cholesterol in a molar ratio of 100:60 by single instillation for a period of 40 days. The left eye was considered as a control in all the experimental rabbits. The rabbits were sacrificed after 40 days. The eyes were separated and histological examination was completed after photographing the cornea vertically.

RESULTS AND DISCUSSIONS

The multilamellar vesicles of ofloxacin were prepared with span 60 and cholesterol in various molar ratios which were selected based on the report of Yongmei Hao et al[14]. The prepared non ionic surfactant vesicles were observed under optical microscope to examine the shape and size of the vesicles and photographed at 400x magnification. Fig.1. shows the morphological character of span 60 and cholesterol in molor ratio of 100:60 which were spherical in shape and slightly larger in size. This may be due to the span 60 with single alkyl tail formed vesicular structure since they have relatively large hydrophobic moieties with low water solubility. Addition of cholesterol into the surfactants achieved suitable molecular geometry and hydrophobicity for bilayer vesicle formation. It has been widely known that the diameter of the vesicles is dependent on the length of the alkyl chain of the surfactants. Generally, the surfactants with longer alkyl chains produced larger vesicles[18].

The amount of entrapped ofloxacin was studied for all the six formulations. The increase of cholesterol into niosome was found to increase the encapsulation efficiency of ofloxacin up to an optimum concentration of cholesterol. The percentage entrapment efficiency of surfactant, cholesterol in molar ratio of 100:30, 100:40 and 100:50 were 49.01%, 60.44% and 70.08% respectively. 73.15% of drug entrapped was observed with the surfactant, cholesterol in molar ratio of 100:60 whereas 100:70 and 100:100 produced 61.55% and 50.01% respectively, Fig.2. These results may be explained by the fact that the long chain surfactant produces high entrapment[14]. The longer alkyl chain influences the HLB value of the surfactant mixture which by its turn directly influences the drug entrapment efficiency[19]. The lower the HLB of surfactant the higher will be the drug entrapment efficiency and stability[8]. Span 60 has the HLB value of 4.7 and highest phase transition temperature of 45ºC. The larger vesicle size may also contribute to the higher entrapment efficiency[18,20]. The increase of cholesterol content 100:30 to 100:60 increases the entrapment efficiency but further increase of cholesterol reduces the entrapment efficiency. This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bilayered structure leading to loss of drug entrapment[21].

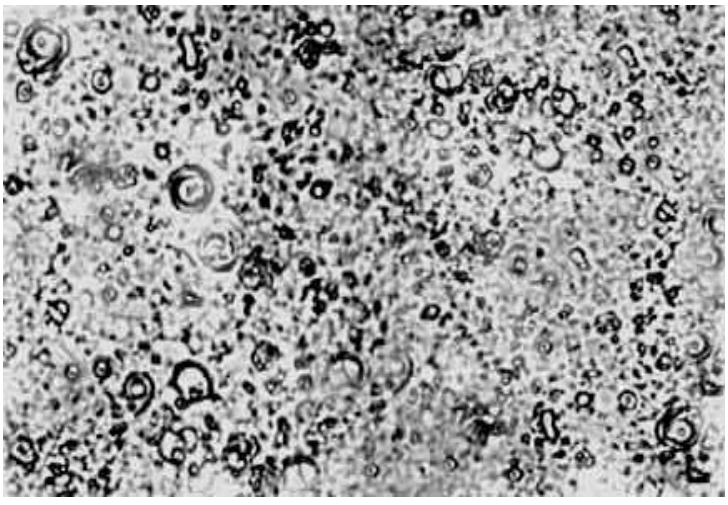

Fig.1: Photomicroscopy of ofloxacin niosome in 100:60 molar ratio

When the drug release study of ofloxacin niosomes was carried out with all formulations for the period of 10h, most of the formulations were found to have a linear release and the formulations were found to provide approximately 60% release. When the cholesterol level was increased in the formulations, 100:30 to 100:60, found to produce the release of 56.11% to 73.77% over the period of 10h. The surfactant, cholesterol in molor ratio of 100: 70 and 100:100 were found to sustain the drug release due to high cholesterol ratio, Fig. 3. These results may be explained by the fact that an increase of cholesterol content resulted in an increase of microviscosity of the membrane indicating more rigidity of the bilayers[18]. Cholesterol has the ability to cement the leaking space in the bilayer membranes[22]. Cholesterol is known to abolish the gel to liquid phase transition of niosome systems[23] which could be able to effectively prevent leakage of drug from niosomes[24]. From the above study, the surfactant, cholesterol in the molar ratio of 100:60 was selected for the further study. Linear regression analysis for the release data was done to determine the proper order of release. Zero-order, first order, Higuchi and Peppas diffusion controlled model equations were applied to all in vitro release results. The results showed the release of diffusion controlled mechanism[25].

The zeta potential probe (DT- 300) was used to study the surface charge analysis of formulation 100:60 and found +26. This shows the practical application in the stability of systems containing dispersed particles since this potential governs the degree of repulsion between adjacent, similarly charged, dispersed particles. This may be explained that the repulsive forces exceed the attractive forces is not favor for flocculation/ aggregation followed by more stable product.

The viscosity of ophthalmic products is generally agreed that an increase in vehicle viscosity increases the residence time in the eye, although there are contradictory reports in the literature to support the optimal viscosity for ocular bioavailability products formulated with a high viscosity are not well tolerated in the eye, causing lacrimation and blinking until the original viscosity of the tear is regained. Drug diffusion out of the formulation into the eye may also be inhibited due to high product viscosity. Finally, administration of high viscosity liquid products tends to be more difficult. The niosomal suspension of surfactant, cholesterol in molar ratio of 100:60 was found to have an optimum viscosity of 1.20cps to prolong the residence time in the eye, compared to solutions and will not produce lacrimation or blinking or blurred vision.

Stability study of surfactant, cholesterol in molar ratio of 100:60 was carried out over the period of 60 days to investigate the leaching of drug from niosomal suspension at refrigerated temperature of 2-8^{0}C. The efficiency of the formulation after storage for the period of 60 days was 79.69%. When the samples were analyzed at specific time intervals, the percent retained were 96.61 at 15th day, 93.83 at 30th day and 89.54 at 45th day. The leakage of drug from for-

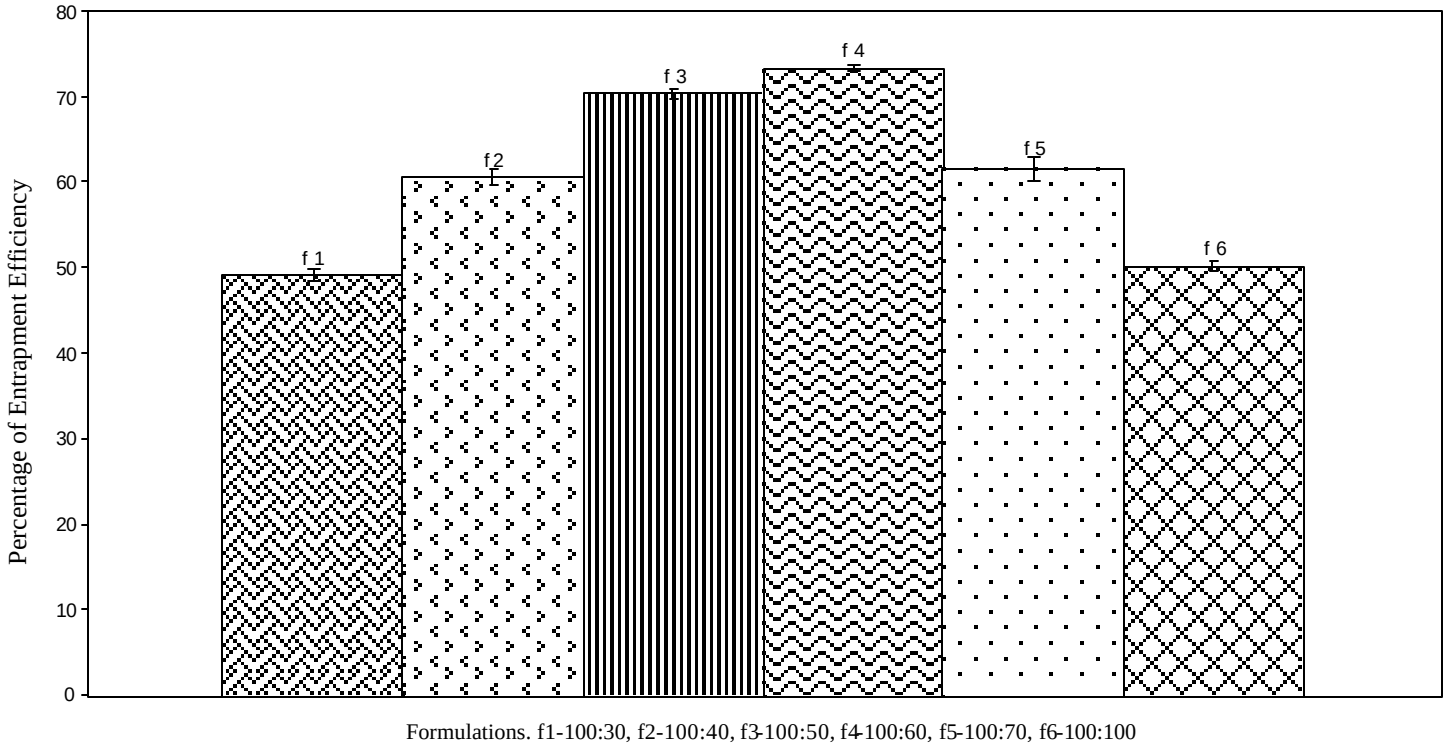

Fig.2: Entrapment efficiency of ofloxacin niosome in 100:60 molar ratio.

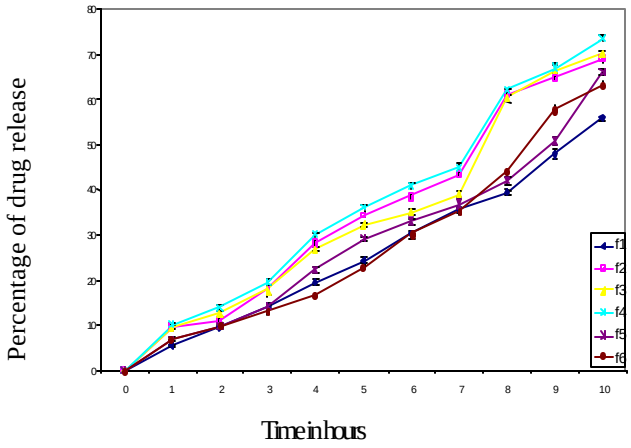

Fig.3: Cumulative percentage of drug release from niosomal suspensions

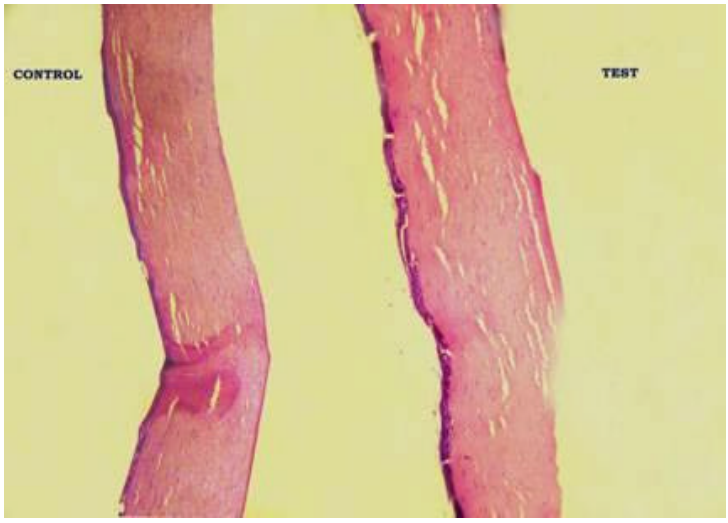

Fig.4: Histological examination of cornea

mulation may be due to its higher surfactant content and least cholesterol level which formed leaking vesicles. Also the results indicate that approximately 90% of ofloxacin was retained in niosomal formulations for a period of 45 days. The system in this study showed that vesicles are stable enough to store under refrigeration temperature with least leakage.

The minimum inhibitory concentration of ofloxacin was found to be 1.75µg/ml for Staphylococcus aureus, 2.50µg/ml for Staphylococcus epidermidis, 2.25µg/ml for Streptococcus pneumonia and 4.25µg/ml for Pseudomonas aeroginosa. This is the lowest concentration at which there was no growth of bacteria as indicated by no turbidity. This result was coincided with the report of Osato et al[26].

The prepared niosomal suspension of ofloxacin in molar ratio of 100:60 was studied to investigate the ocular availability of drug for the prolonged action after the single dose. The study carried out by comparing the aqueous humor extract samples of test with control. The concentrations of ofloxacin niosomes were higher than the ofloxacin control. The retention time was 5.53. The C_{max} of 4.373µg/ml, T_{max} 200 mins and AUC 425.294 were observed for ofloxacin niosome. 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. The surfactant in the formulation

acts as penetration enhancers as they can remove the mucus layer and break junctional complexes[27-30]. The concentrations of ofloxacin present in aqueous humor in test eyes were higher than the control and above the MIC level. The control produced the C_{max} at 2.9575µg/ml, T_{max} 80 min and AUC 303.99. This may be due to less, l-3%, availability of drug for the absorbance through corneal route. The t-test revealed significant differences at $P<0.001$.

The potential ocular irritancy effect of the formulations under test was evaluated by observing them for any redness, inflammation, or increased tear production. The control and test corneal tissues, following instillation of multi-lamelar niosomal formulation composed of span 60 and cholesterol in a 100:60 molar ratio on six rabbits were observed. Both eyes of the rabbits under test were examined for any signs of irritation. When compare with control, the suspension under test did not show any sign of redness, inflammation or increased tear production. Histopathology of cornea of both samples was showed slightly oedema of the substantia propria especially in the deep stroma where the collagen fibers were separated from each other, Fig. 4. This change may be due to slight irritation caused by the nonionic surfactant, Span 60[31]. This type of irritation is reversible where the slight oedema clears over time. As a conclusion, no major changes are observed, and therefore the preparation may be considered to use for the short and long term treatments.

CONCLUSIONS

Ofloxacin ophthalmic solution is indicated for the treatment of infections caused by susceptible strains of staphylococcus aureus, staphylococcus epidermidis, streptococcus pneumoniae, enterobacter cloacae, haemophilus influenzae, proteus mirabilis, pseudomonas aeruginosa and propionibacterium acnes. Conventional dosage forms of ofloxacin will not produce effective absorption for the prolonged period of time due to non availability of drug in the absorption site. Niosomal suspension formed with span 60, cholesterol in a molar ratio of 100:60 showed highest drug entrapment of 73.15% and produced 73.77% drug release in-vitro over the period of 10h. Zeta potential, +26, conform the stability. Viscosity was 1.20cp which showed suitable consistency for ocular preparation and did not produce blurred vision or drainage. The niosomal formulation also found to ensure a good ocular bioavailability of the drug in-vivo with 4.373 µg in aqueous humor. By these facts, study can be concluded by saying niosomes formed with span 60, cholesterol in the molar ratio of 100:60 is a promising approach to improve the bioavailability of ofloxacin for an extended period of time.

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