

LECITHIN-POLOXAMER BASED NANOFOMULATION FOR OPHTHAMIC DELIVERY

Bhagwat Lodhi, Priyanka Rathore, Surendra Jain, Durga Pandey*

Sagar Institute of Research and Technology Pharmacy Bhopal, Pin-462033, Madhya Pradesh, India.

*Corresponding Author: Durga Pandey

Sagar Institute of Research and Technology Pharmacy Bhopal, Pin-462033, Madhya Pradesh, India.

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ABSTRACT

Nanoemulsion can be defined as oil-in-water (o/w) emulsions with mean droplet diameters ranging between 100 and 500 nm, terms sub-micron emulsion and mini-emulsion are used as synonyms. The aim of the present study is to investigate the potential of a nanoemulsion formulation for ocular delivery of Ofloxacin. Our aim is to develop and characterize the nanoemulsion bearing antimicrobial drug for improvement of drug absorption from precorneal area. Our developed delivery system will result with improved drug retention in precorneal area and antimicrobial efficacy. Oil in water nanoemulsion prepared by using lecithin as surfactant and pluronic F-68 as co surfactant with the help of high shear stirring. Formulation optimized on the basis of their size and stability. FTIR confirmed that drug is Ofloxacin. Melting point of drug was found 246-248°C, Particle size was found less than 500 nm, Viscosity of optimized formulatipon was 3. pH was 7.1 Entrapment of drug was found ~70%. Sustained release formulation was achieved with 59.43 % release in 24 hrs. Formulaton was observed visually for phase preparation and creaming and found stable up to three months. No phase separation observed.

KEY WORDS: Nanoemulsion, in vitro release, sustained drug release, ocular.

INTRODUCTION

Ocular administration of drug is primarily associated with the need to treat ophthalmic diseases. Eye is the most easily accessible site for topical administration of a medication. Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time.^[1] Topical application of drugs to the eye is the well established route of administration for the treatment of various eye diseases like dryness, conjunctiva, eye flu etc. The protective mechanisms of the eye such as Blinking, baseline and reflex lachrymation, and drainage decrease the bioavailability of drug and also help to remove rapidly foreign substances like the dust particles bacteria, including drugs, from the surface of the eye.^[2] There are many eye diseases which can be affected to the eye and also eye vision. They are treated by conventional formulation. But these preparations when instilled into the eye they are rapidly drained away from precorneal area.

There are most commonly available ophthalmic preparations such as drops and ointments about 70% of Reason for precorneal drainage due to blinking, tear flow, lacrimal and nasal drainage of the eye. Only a small amount of drug is available for its therapeutic effect resulting in frequent dosing application to the eye. With conventional ophthalmic solution normal dropper

used which delivers about 50-75 μ l per drop and portions of these drops rapidly drain until the eye is back to normal resident volume of 7 μ l. Due to this drug loss in front of the eye, very small drug is available to enter the cornea and inner tissue of the eye.

Actual corneal permeability of the drug is relatively low and very small corneal contact time (about 1-2min) in humans for instilled solution usually less than 10%. Due to these limitations, controlled drug delivery to the eye is restricted imposed by the efficient protective mechanism.^[3-5] So overcome to these problems newer pharmaceutical ophthalmic formulation such as in-situ gel, nano-particle, liposome, Nano-suspension, submicron emulsion, ionphoresis and ocular inserts have been developed in last three decades increase the bioavailability of the drug as a sustained and controlled manner.

The aim of the present study was to investigate the potential of an emulsion formulation for ocular delivery of Ofloxacin. Our aim is to develop and characterize the nanoemulsion bearing antimicrobial drug for improvement of drug absorption from precorneal area. Our developed delivery system will result with improved drug retention in precorneal area and antimicrobial efficacy. Nanoemulsion is a novel vesicular system for the delivery of drugs in to the ocular route. The use of

emulsion improved penetration of active constituent in the body. Nanoemulsion gives more flexibility for the delivery of the drug. Nanoemulsion gives controlled release of drugs. Greater stability of active pharmaceutical ingredients. Nanoemulsion has better biocompatibility. Decrease the tolerance of antibiotic drug. As compare to conventional ocular dosage form nanoemulsion gives better absorption. Long duration of drug for a particular amount of drug reduces the dosage frequency. Patient compliance, reduce side effects.^[6-7]

Advantage of nanoemulsions

1. Nanoemulsion has higher surface area and free energy that make them an effective transport system.
2. They do not show the problems of inherent creaming, flocculation, coalescence and sedimentation.
3. It can be formulated in variety of formulations such as foams, creams, liquids and sprays.
4. They are non-toxic; non-irritant hence can be easily applied to the skin and mucous membranes.
5. It can be administered orally if the formulation contains surfactants which are biocompatible.
6. It do not damage healthy human and animal cells hence are suitable for human and veterinary therapeutic purposes.
7. It provides better uptake of oil-soluble supplements in cell cultures technology to improve growth of cultured cells and allows toxicity studies of oil-soluble drugs.
10. It constitutes the primary step in nanocapsules and nanospheres synthesis using nano precipitation and the interfacial poly-condensation.^[8-9]

MATERIALS AND METHODS

Ofloxacin received as gift sample Astam health care PVT. Ltd (Baddi, India). Pluronic F-68, Pharma gift sample grade. Pluronic F-68, Pharma gift sample, Soya lecithin Pharma, SIRT-P, Soya oil Pharma SIRT-P. Preformulation studies for selected drug Ofloxacin include physical appearance, melting point, identification, solubility profile and determination of partition coefficient.

- a) **Physical appearance:** White to off-white powder.
- b) **Melting point determined by capillary method**
Capillary melting gives information about the melting range but it is different to assign an accurate melting point.
- c) **IR-Spectra:** Identification of drug was done by IR Spectra. The infrared spectral assignment of Ofloxacin was obtained by using KBr using in Prestige Spectroscopy.
- d) **Determination of pH of 1% solution**
gm of powder was taken and dissolved in 100 ml of distilled water with sonication and filtered. pH of the filtrate was checked with electronic pH meter. The solubility of Ofloxacin was tested in various solvent. A definite quantity (5mg) of drug was dissolved in 10 ml of each investigated solvent at room temperature. The solubility was observed only by the UV Spectrophotometric.
- e) **Quantitative estimation of drug**
- f) Absorption maximum was determined by shimadzu-1700 UV/visible spectrophotometer, which exhibits maxima at 287 nm.

UV Spectrophotometric method based UV/visible spectrophotometer of Ofloxacin was used to estimate the drug concentration in the range of 2-10 µg/ml in phosphate buffer saline (PH 7.4) as it follows the beer's law in this range.

5 mg accurately weighed Ofloxacin was dissolved in 5 ml of PBS (pH 7.4) and volume made up to 10 ml with PBS (pH 7.4) in volumetric flask (10ml) stock-A). 1 ml of stock solution- A was diluted up to 10 ml with PBS (pH 7.4) (Stock-B). From stock B 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml were taken then volume was made upto 10 ml with PBS (pH 7.4). This gives the concentration of 2, 4, 6, 8, 10 µg/ml respectively. Absorbance was measured in SHIMADZU 1700 UV/ visible spectrometer at λ_{\max} 287 nm against blank and observation recorded and calibration curve was plotted.

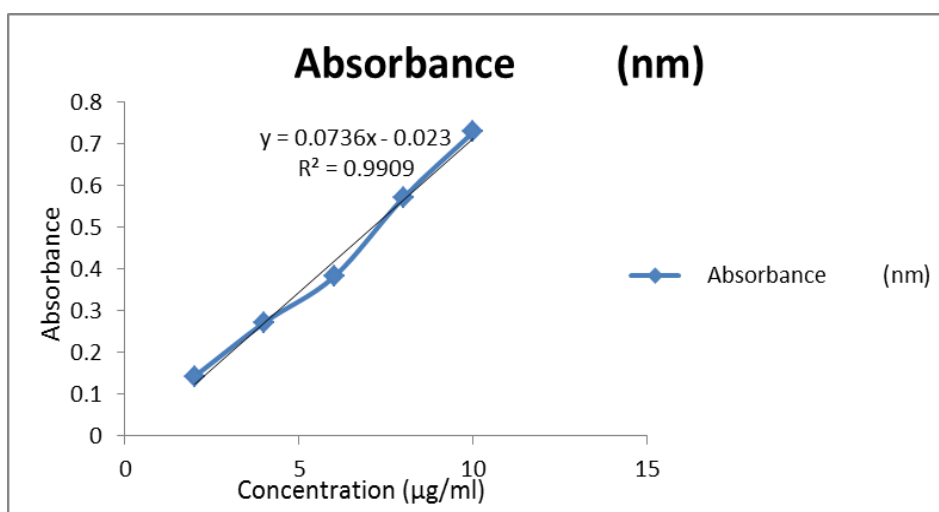


Figure-1 Calibration curve of Ofloxacin in PBS (7.4).

Preparation of nanoemulsion

Different nanoemulsion containing Ofloxacin NE-OF. The lipid phase was prepared by heating soya oil at 60°C containing lecithin. The NE-OF (equivalent to 0.3% OF) was added to the lipid mixture mentioned above. The lipid phase was added gradually to aqueous phase containing pluronic F-68 at 60° and magnetically stirred. The primary emulsion was prepared by stirring for 20 min using high shear mixer at the speed of 16000, and subsequent emulsification was accomplished by sonication using ultrasonic probe at 20% amplitude for 8 min.^[10]

Physicochemical Characterization

Microscopy of optimized formulations

Formulation was measured microscopically from an optical microscope (Cippon, Japan) used to observe the shape of the prepared formulation droplets fig-5.

Particle size

The particle size was measured by a zetasizer 2000 (Malvern instruments). A sample was extemporaneously diluted in distilled water to an appropriate concentration before measurement at room temperature. The measurements were carried out in the fully automatic mode. Each sample was analyzed twice fig-6.

pH

The pH meter was used for the determination of the pH value of the emulsion at room temperature (25°C ± 2).

Drug entrapment

The OF loaded emulsion were centrifuged at 17000 and 4°C for 30 min in eppendorf centrifuge in order to separate the incorporated drug from the un-incorporated drug. The supernatant was analyzed by UV spectroscopy for the un-incorporated drug (A1) concentration to determine the encapsulation percentage from total amount of drug (A2). Entrapment efficiency was calculated using the equation^[10]:

$$EE (\%) = (A2 - A1 / A2) \times 100$$

In-vitro drug release (dialysis method)

In vitro release studies were carried out using dialysis bag technique at 37°C. For the release experiment, 1ml of NE containing OF and its ionic complex was pipetted into a dialysis bag. The dialysis bag was kept in 100 ml of stirred sink solution (PBS, pH 7.4) for 24 hours, temperature was maintained at 37°C. The samples were collected at different time durations i.e. 0, 15, 30, min, 1 hr, 2hr, 4hr, 6hr, 12hr and 24hr the release medium was exchanged with equal volume of fresh PBS solution. The concentration of released OF was assayed by UV spectroscopy SHIMADZU 1700.^[11]

Process variables

Formulation of nanoemulsion can be affected by number of factors (process variables) which are directly affecting the properties of the Nanoemulsion. The preparation of

Nano emulsion involves various process variables, but out of them, following were studied:-

- Effect of homogenizer- formulations were subjected to different speed 1500, 8000, 16000 rpm.
- Effect of sonication time- formulations were subjected to different sonication time i. e. 2 min 5 min and 8 min.
- Effect of temperature formulations were subjected to different temperature 40, 50 and 60 and observed for any change in globules size with respect to temperature.

Stability study: - Formulation was stored at 4°C and 25°C for 3 month. The creaming and the phase separation were assessed visually at given time intervals.

RESULTS AND DISCUSSIONS

Melting point of drug was found 246-248°C and pH of drug was 7.5 determined as average of three determinations table-1. FTIR confirmed that drug is Ofloxacin.

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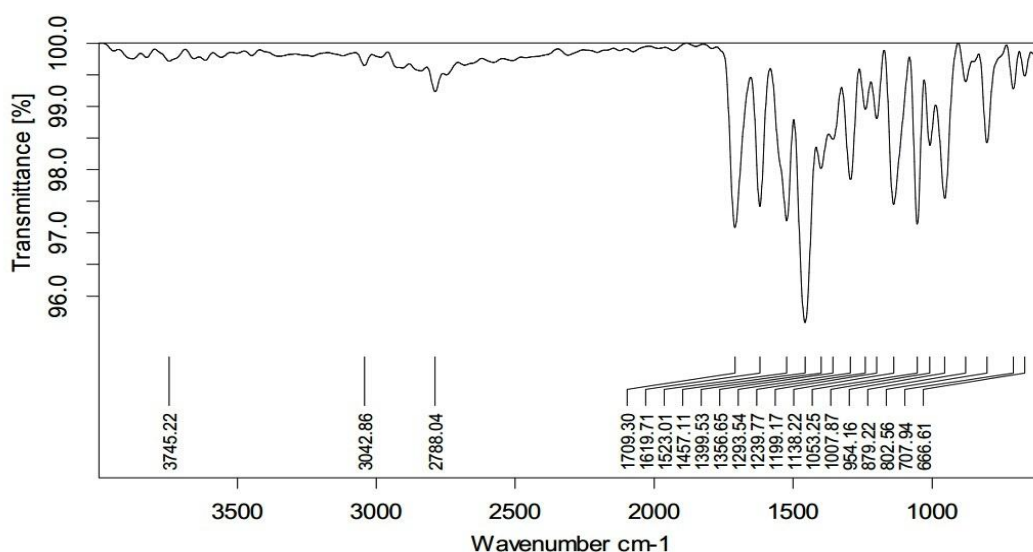


Figure-2 FTIR OF OFLOXACIN.

FTIR Interpretation of Drug

The carboxylic acid group (OH) band in the region of 3745 cm^{-1} . The peak at 1709 cm^{-1} showing the carbonyl stretching ($\text{C}=\text{O}$) of carboxylic acid and 1619 cm^{-1} peak may be due to $\text{C}=\text{C}$ stretch at C-2 and C-3 position. Other peaks are the 1399 cm^{-1} which is the vibration associated with the protonation of N_4 at piperazinyl group, 1523 cm^{-1} which corresponds to the $\text{C}=\text{O}$ aromatic stretching and 1053 cm^{-1} which corresponds to the $\text{C}-\text{O}-\text{C}$ stretching of the ether group.

Formulation optimized on the basis of stability and particle size. Different formulations prepared by changing the percentage of surfactant, co-surfactant and oil percentage. We selected lecithin as surfactant and pluronic F-68 as co-surfactant combination of both required to produce a stable formulation. The effects of variables were observed on the final particle size, drug loading during the preparation of a particular system, the other variables were kept constant. The observations are shown in table-1.

Table-1: Process variables.

S. NO.	Formulation code	Speed (rpm)	Particle stability	Sonication time (min)	Particle size (nm)	Temperature $^{\circ}\text{C}$	Percentage entrapment
1.	NE-OF	16000	Stable	2	1000.6	60	72.3%
2.	NE-OF	8000	Unstable	5	650.4	65	63.5%
3.	NE-OF	1500	Unstable	8	250	70	58.2%



Figure-3 Ofloxacin Nanoemulsion.

Table-2: Characterization of optimized formulation.

Formulation	Average particle size (nm)	pH	Viscosity
NE	236.5 \pm 30	7.2 \pm 0.4	2
NE-OF	245 \pm 20	7.1 \pm 0.2	3

Drug entrapment

The entrapment efficiency was found to be more than ~70 % in optimized formulations table-1.

compared to control. The control group used was a dispersion of drug in aqueous media.

In-vitro drug release (dialysis method)

It has been revealed from the graph that the drug release by NE-OF formulation was in sustained manner

Table-3: Drug release from optimized OF formulation.

Time (hrs)	OF (control)	NE-OF (Formulation)
0	0	0
0.15	83.2	15
0.3	99	24
1	83	31.6
2	72	35.5
4	59	40.1
6	45	48.2
12	32	55
24	10	59.43

NE-OF- Nanoemulsion containing Ofloxacin
 OF- Dispersion of drug in distilled water

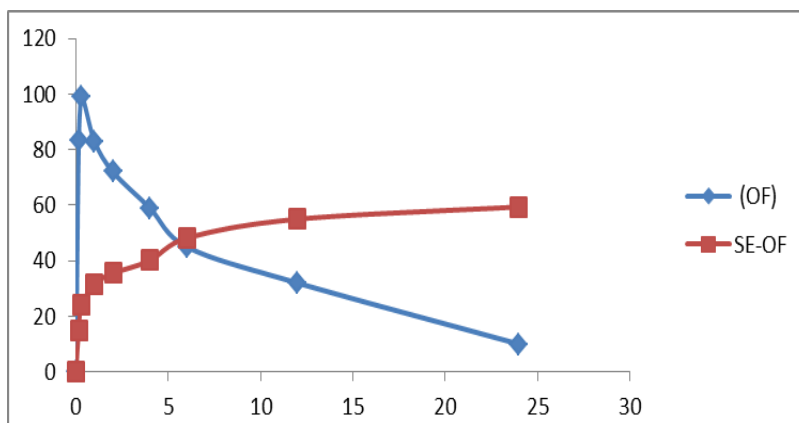


Fig-4 In vitro release profile of OF loaded nanoemulsion formulations carried out using bulk equilibrium reverse dialysis bag technique at 37°C.

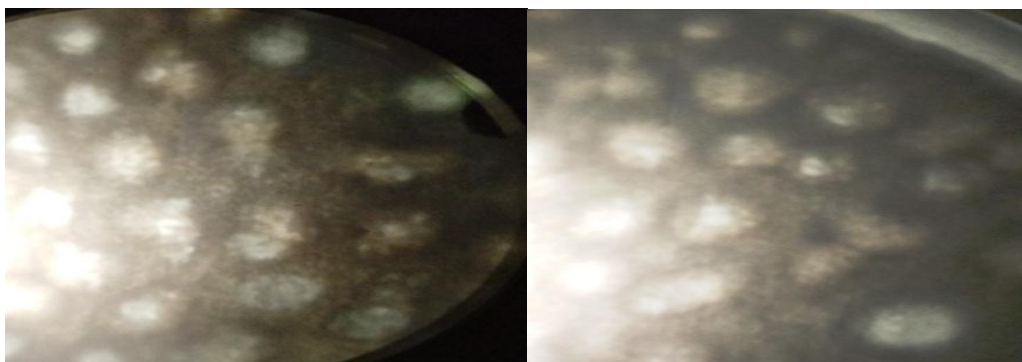


Fig-5 Microscopic Images of Optimized Nanoemulsion.

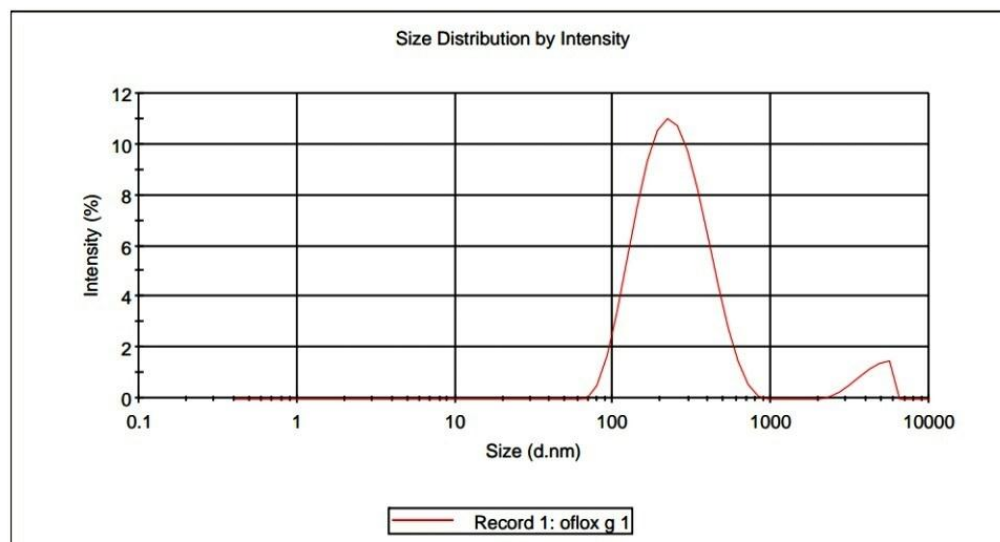


Figure – 6: Particle size of nanoemulsion droplets.

Stability studies

There was not any phase separation and creaming observed in optimized formulation upto three months of storage.^[14-16]

CONCLUSION

In summary, we can concluded that Ofloxacin is more effective when given as nanoemulsion as compared to conventional ocular dosage form. Ofloxacin is a new broad-spectrum antimicrobial drug is highly effective in treating various types of ocular infections. Patients with infections caused by a mixture of organisms, and those allergic to P-lactam antibiotics, may be appropriate candidates for treatment with ofloxacin. From this study it is concluded that the nanoemulsion have increased drug absorption from ocular route. In this way nanoemulsion proved to be an effective dosage form for the treatment of infectious diseases and improving the bioavailability of Ofloxacin as compare to other dosage form. Optimized formulation was good in respect of particle size, viscosity, stability and P^H . Release was also in sustained manner, entrapment was more than 70 % in optimized.

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CONFLICT OF INTEREST

Declared None.

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