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Original Research Article

Niosomes encapsulated with Gatifloxacin for ocular drug delivery

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Abstract

The objective of the present research was to formulate and evaluate non-ionic surfactant vesicles (niosomes) as carriers for the delivery of Gatifloxacin. Niosomal formulations were prepared using surfactant Span 60 in the presence of cholesterol and stearylamine (SA) in different molar ratios by a thin film hydration technique using rotary evaporator. The five batches of niosome formulations were prepared in the ratios 1:0.5:0.1, 1:1:0.1, 1:1.5:0.1, 1.5:1:0.1, 0.5:1:0.1 (surfactant: cholesterol: SA). Niosome prepared by with ratio 0.5:1:0.1 provided lower entrapment efficiency (49.65%) while that of 1:1:0.1 provide most advantageous entrapment efficiency (65.12%). *In-vitro* drug release results confirmed that niosomal formulations have a high retention of Gatifloxacin ($Q_8h=71.45\%$) the vesicles. Antimicrobial efficacy study revealed equal effectiveness of drug in niosome and aqueous eye drop. The formulation was isotonic hence non irritant to ocular mucosa. Ocular irritancy test performed on albino rabbits, showed no sign of irritation for all tested niosomal formulations.

KEY WORDS: Niosomes, Gatifloxacin, Ocular drug delivery

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1.0 Introduction

In recent years, vesicles have become the vehicle of choice in drug delivery. Vesicles can play a major role in modeling biological membranes and in the transport and targeting of active agents. Vesicles offers a promising avenue not only to fulfill the need for the ophthalmic drug delivery system that has the convenience of a drop but also will localize and maintain drug delivery at its site of action. Vesicular drug delivery system used in ophthalmic broadly includes liposomes and niosomes. Niosomes are formations of vesicles by hydrating mixture of cholesterol and nonionic surfactants [1]. These nonionic surfactants vesicles are called niosomes. These are formed by self-assembly of non-ionic surfactants in aqueous media as spherical, unilamellar, multilamellar system and polyhedral structures in addition to inverse structures which appear only in non-aqueous solvent [2,3]. Niosome are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures which can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in vesicular membrane [4]. Niosomes in topical ocular delivery are preferred over other vesicular systems because of the following reasons: (1) chemical stability; (2) low toxicity because of their non-ionic nature; (3) handling surfactants with no special precautions or conditions; (4) the ability to improve the performance of the drug via better availability and controlled delivery at a particular site; (5) being biodegradable, biocompatible and non-immunogenic [5]. Various studies have demonstrated the successful use of niosomes as ocular drug delivery carriers where these vesicles

significantly improved the ocular bioavailability of cyclopentolate, with respect to reference buffer solution. No irritation with the niosomal formulation was observed Saettone et al., 1996 reported that there was approximately a 2.5 times increase in the ocular bioavailability of timolol maleate (a water soluble drug) encapsulated in niosomes as compared to timolol maleate solution [6]. An increased ocular bioavailability of water-soluble drugs, entrapped in niosomes, may be due to the fact that surfactants act as penetration enhancers by removing the mucus layer and breaking junctional complexes [7, 8].

Gatifloxacin a synthetic broad-spectrum antimicrobial fluoroquinolone, that is active against both gram-negative and gram-positive bacteria including *S. aureus*, is used in the treatment of eye, in a wide range of infections such as conjunctivitis, keratitis and keratoconjunctivitis. The anti-bacterial activity of gatifloxacin result from inhibition of DNA gyrase (topoisomerase II) and topoisomerase IV, the enzyme that plays a key role in partitioning of the chromosomal DNA during bacterial cell division. It is presently available as eye drops 0.3% and administered at dosing interval of one drop every two hours in the affected eye in treatment of bacterial conjunctivitis caused by susceptible strains of the following aerobic gram- positive and gram- negative bacteria such as *S. aureus*, *S. epidermidis*, *S. pneumoniae*, *S. mitis*, *Comyebacterium propinquum* and *Pseudomonas aeruginosa* [9].

The purpose of the current study was to prepare gatifloxacin encapsulated niosomes possessing a high drug loading capacity in order to be used as ophthalmic carriers for

topical ocular infections. Vesicle dispersions were characterized by transmission electron microscopy for vesicle formation and morphology. All formulations were evaluated for vesicular size, entrapment efficiency, *in vitro* drug release and optimized finally. An ocular irritancy test performed on albino rabbits was done in order to evaluate the optimized formulations upon application to the eye.

2.0 Materials and methods

2.1 Materials

Gatifloxacin was obtained as gift sample from Matrix Laboratories limited, Mumbai India. cholesterol from Molychem company, Mumbai and Span 60 was purchased from Research lab Fine Chem Industries, Mumbai (India), Diethyl ether and 1 propanol, 1,2 Propanol were from Merck specialties private ltd, worli, Mumbai, stearylamine (SA) from sigma Aldrich, USA.

2.2 Methods

Preparation of Gatifloxacin Niosomes

Niosomes containing gatifloxacin were prepared by thin film hydration technique [10, 11]. The surfactants, cholesterol and stearylamine (SA), in different molar ratios(**Table1**), were accurately weighed into a long necked quick fit round-bottom flask (500 ml) and dissolved in 15 ml Diethyl ether. The organic solvent was slowly evaporated at 60 °C under reduced pressure, using a rotary evaporator (Buchi R-215 Rotavapor, Switzerland) at 100-150 rpm such that a thin dry film of the components was formed on the inner wall of the rotating flask. The dried thin film was

$$\text{Entrapment efficiency (\%)} = \frac{\text{amount of entrapped gatifloxacin}}{\text{Total amount of gatifloxacin}} \times 100$$

Transmission Electron Microscopy

Gatifloxacin niosomal formulations were characterized by transmission electron microscope (JEM-1230, Jeol, Tokyo, Japan) at 70 kV, after being negatively

then hydrated with 10 ml of phosphate buffered saline (PBS, pH 7.4), containing 10 mg gatifloxacin, by rotating the flask in a water bath using a rotavapor under normal pressure in order to ensure complete hydration of the film. The niosomal suspension was left to mature overnight at 4 °C. All above mentioned steps were done under aseptic conditions in order to maintain sterility. All glassware was sterilized by autoclaving, phosphate buffered saline was passed through a 0.22 µm membrane filter. Formulations were sonicated in a bath sonicator for 30 min. All Vesicle suspensions were also sonicated for 5 min.

Characterization of Niosome

Determination of Entrapment Efficiency (%EE)

The amount of encapsulated gatifloxacin was obtained by ultra centrifugation of one ml of the niosomal suspension at 10,000-15,000 rpm for 1.5 h using a cooling centrifuge at 4 °C. The niosomes were separated from the supernatant and were washed thrice (3X), each time with 1 ml phosphate buffered saline, and recentrifuged again for 1 h. The amount of entrapped gatifloxacin was determined by lysis of the separated vesicles with isopropanol. About 100 µl sample of niosomes was mixed with 1 ml of isopropanol; the volume was made up to 10 ml with phosphate buffered saline [12]. The concentration of the drug was determined spectrophotometrically at 287 nm (UV spectrophotometer, 1700, Shimadzu)

The entrapment efficiency is defined as follows:

stained. Briefly to an aliquot of a suspension of prepared niosome formulation, sufficient quantity of saturated aqueous solution of ammonium molybdate on to the carbon coated grid. The grid was allowed to dry and

it was observed under transmission electron microscope.

Determination of Vesicle Size

The average z diameter of sonicated vesicles was determined by dynamic light scattering using zeta sizer, Nano ZS 90 (Malvern Instruments Ltd). For measurement, 100 μ l of the formulation was diluted with an appropriate volume of PBS (pH 7.4), and the vesicle diameter, polydispersity index were determined.

Zeta potential measurement

Zeta potential was analyzed using zeta sizer, Nano ZS 90 (Malvern Instruments Ltd) to measure the stability of niosome by studying its colloidal property. The measurements were performed after dilution with distilled water at room temperature [13].

In Vitro Release from Niosomes

In vitro release was studied using a dialysis bag (Dialysis membrane, 12,000-14,000, mol. wt cut-off) as a 'donor compartment'. Niosomes containing entrapped gatifloxacin obtained after centrifugation of 2 ml of the formulation were resuspended in 1 ml of PBS, pH 7.4, and used for the release study. The dialysis membrane was soaked in warm water for 10 min, one end was sealed with a clip, the niosome preparation or free gatifloxacin solution was pipetted into the bag and the bag was sealed with another closure clip to prevent leakage. The dialysis bag was placed in 100 ml of PBS, pH 7.4, at $37 \pm 2^\circ\text{C}$. The medium, which acted as the receptor compartment, was stirred at 100 rpm. Samples of medium (5 ml) were withdrawn hourly and replaced with fresh buffer for period of 8 h. Drug content was determined spectrophotometrically at 287 nm using PBS as blank [14].

Antimicrobial Efficacy Studies

Antimicrobial efficacy studies were carried out to ascertain the biological activity of niosome formulations against microorganisms. This was determined by

agar diffusion test employing "cup plate technique" as per Indian Pharmacopoeia 1996. Sterile solution of gatifloxacin (Gatiquin[®] eye drops, Cipla India.) as a standard was used and the niosome formulation (F2) as a test formulation were poured into cups bored into sterile Muller Hinton Agar (MHA) previously seeded with test organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). After allowing diffusion of solutions for two hours, the plates were incubated at $37 \pm 0.5^\circ\text{C}$ for 24 hr. The zone of inhibition (ZOI) measured around each cup was compared with that of the standard. Both positive and negative controls were maintained throughout the study [15].

Isotonicity evaluation studies

Isotonicity is important characteristic of the ophthalmic formulation. Isotonicity has to be maintained to prevent tissue damage or irritation of eye. Formulation (F2) was subjected to isotonicity testing, since it exhibited good release characteristics. Formulation was mixed with five drops of blood, observed under microscope at 45X magnification, for the shape of blood cells and compared with standard marketed ophthalmic formulation (Gatiquin[®] eye drops) containing Gatifloxacin [16].

Ocular Irritancy Test

The animal experiment was carried out in compliance with the protocol of the Institutional animal ethical committee (Registration No: 651/02/C/CPCSEA under CPCSEA, India). Six New Zealand white rabbits with mean weight of 2.5 ± 0.3 kg were used. The rabbits were accommodated to the dosing for 1 month before the study to prevent withdrawal and defense reaction that may lead to inaccurate dosing. The rabbits were kept in a single cage and fasted for 12 h before the study with free access to water during the experiments. The potential ocular irritancy and/or damaging effects of

niosome formulation (F2) was evaluated by observing them for any redness, inflammation, or increased tear production, upon application to the eyes of albino rabbits. Formulation was tested on three albino rabbits; the experiment was performed by a single instillation (50 μ l) of the niosomal preparation under test into the conjunctival sac of one eye, while the contra lateral eye served as control. Both eyes of the rabbits under test were examined for any irritation signs, such as conjunctival: corneal edema and/or hyperemia on the basis of direct visual observation using a slit lamp, before treatment, and 1, 24 and 48 h after instillation. Similar procedure was carried on three albino rabbits for standard formulation (Gatifloxin[®] eye drops) [17, 18].

Stability studies

The prepared formulations were tested for stability by storing them at $25 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH. Formulation was evaluated for residual drug content by taking samples at 30th, 60th and 90th day and estimated by spectrophotometric method, at 287 nm.

3.0 Results and discussion

Entrapment Efficiency (%EE)

The entrapment efficiencies of all niosomal formulations are reported in **Table 2**. Cholesterol is one of the common additives included in the formulation in order to prepare stable niosomes. Cholesterol stabilizes bilayers, prevents leak, and retards permeation of solutes enclosed in the aqueous core of these vesicles. Cholesterol is known to abolish the gel to lipid phase transition of niosome systems, which could be able to effectively prevent leakage of drug from niosomes. Cholesterol is thus usually included in a 1:1 molar ratio (non-ionic surfactant: cholesterol) in most formulations. However even after the addition of cholesterol, the intrinsic phase transition behavior of vesicle forming

surfactants still influences the properties of the dispersion: notably the membrane permeability, encapsulation efficiency and bilayer rigidity. SA, a charged molecule, is often used to prevent niosome aggregation and increase the stability of niosome dispersions. In presence of SA, equal molarity of these non-ionic surfactants and cholesterol showed higher entrapment efficiency than a 1:0.5 molar ratio. This may be due to the fact that cholesterol in the presence of SA was more efficiently able to stabilize the structure of the niosomal membrane in a molar ratio of 1:1 (non-ionic surfactant: cholesterol). It is clear that formula F2 composed of Span 60, cholesterol and SA in a 1:1:0.1 molar ratios is most beneficial for the efficient encapsulation of gatifloxacin as it exhibited the highest entrapment efficiency (65.12 ± 0.7) with better particle size 883 nm compared to the other formulae [19].

Transmission Electron Microscopy (TEM)

TEM images of niosomes shows that niosomes prepared by thin film hydration were well defined and nearly spherical in shape (**Fig 1**).

Determination of Vesicle Size

Niosomal vesicles containing higher amount of cholesterol (F3) and higher amount of span 60 (F4) were larger in size than those with lower amount of cholesterol and span 60 (F2). This suggests that vesicle size increases with increase in surfactant concentration (**Table 2**).

Zeta potential measurement

The optimized formulation (F2) was subjected to zeta potential measurement had a zeta value of -30 mv, which is a measure of net charge of niosomes (**Table 2**). The magnitude of the zeta potential gives an indication of stability. The 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. The formulation F2 had stability at ambient storage condition. Moreover particles with zeta potential close to zero are less able to undergo phagocytosis than charge particles [20].

In Vitro Release from Niosomes

Results of an in vitro study on the release of gatifloxacin niosomal vesicles prepared using span 60, cholesterol, SA are shown in **Fig.2**. The percentage of drug released from the optimized formulation (F2) was 71.45 % within a period of 8 hours. Niosome formulations showed slower release rate of gatifloxacin. Significant changes in release were observed upon changing the amount of surfactant. It was also observed that the increase of cholesterol molar ratio from 1:1:0.1 to 1.5:1:0.1(cholesterol: span 60: SA) reduced drug release from niosome preparation which was result of improvement of bilayer rigidity and stability ability of cholesterol. Hence the release of drug from vesicles was at slower rate over a prolonged period of time.

Antimicrobial Efficacy Studies

Zone of Inhibition of niosome formulation (F2) was found similar with that of the standard formulation of gatifloxacin (Gatiquin[®] eye drops) (**Fig 3**). It reveals that the gatifloxacin was equally effective in same concentration as a niosome as that of aqueous eye drops.

Isotonicity evaluation study

The blood cells were found to be spherical and did not rupture or shrink after mixing with the standard and test formulations. Results indicate both formulations were isotonic with body fluids and hence non-irritant to the rabbit eye (**Fig 4**).

Ocular Irritancy Test

In the present study the rabbits remained conscious throughout the experiment, thereby providing functional eye movement.

None of signs of redness, inflammation or increased tear production was observed over the study period (48 h) for tested formulations (**Fig 5**). This indicated that the non ionic surfactants namely span 60 as well as the other excipients such as stearylamine and cholesterol used in the niosome formulations was non-irritant to the eye could be used safely.

Stability studies

Storage stability was also evaluated in terms of percent residual drug remaining in the vesicle, considering initially drug content as 100%. There was a minimum or no reduction of drug content was found when formulation was stored at $25\pm 2^{\circ}\text{C}$ and $75 \pm 5\%$ RH (**Table 3**).

4.0 Conclusion

Niosomes of gatifloxacin allowed a significant vesicular carrier system for therapeutic effectiveness in terms of duration of action and decrease in dose frequency. The drug release studies showed that there was slow and prolonged release of drug up to 8h from niosome formulation. Niosome suspensions are isotonic with body fluids and non irritant to ocular mucosa. Niosomes are having equal antibacterial activity as that of aqueous solution. Hence niosomes may be considered as promising ophthalmic carriers for the topical application of gatifloxacin. The above findings open new prospects for ocular application. However, more exhaustive preclinical and clinical studies are needed to be performed to provide further information and insight into these approaches.

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Reference

1. Handjani-vila, RM. Dispersion of lamellar phases of nonionic lipids in cosmetic products. *Int J Cosmetic Sci.* 1979; 1: 303.
2. Uchegbu IF and Florence AT. Non-ionic surfactant vesicles (niosomes)-physical and pharmaceutical chemistry. *Adv Colloid Interface Sci.* 1995; 58:1.
3. Murdan S. Inverse toroidal vesicles: the precursors of tubular structures in sorbitan monostearate organogels. *Euro J Pharm Sci.* 1998; 6:22.
4. Carafa M, Santucci E, Alhaique F, Coviello T, Murtas, E, Ricciari, FM, Lucania, G, Torrisi. MR. Preparation and properties of new unilamellar non-ionic surfactant vesicles'. *Int J Pharm.* 1998; 160:51–59.
5. Lucania G, Carafa M, Santucci E. Lidocaine-loaded non ionic surfactant vesicles: characterization and in vitro permeation studies. *Int J Pharm.* 2002; 231:21–32.
6. Saettone MF, Perini GM, Santucci E, Alhaique F. Non-ionic surfactant vesicles as ophthalmic carriers for cyclopentolate. A preliminary evaluation. *S. T. P. Pharma Sci.* 1996; 6:94–98.
7. Vyas SP, Mysore N, Jaittley V. Venkatesan N. Discoidal niosome based controlled ocular delivery of timolol maleate'. *Pharmazie,* 1998; 53:466–469.
8. Green K, Downs S. Ocular penetration of pilocarpine in rabbits. *Arch. Ophthalmol.* 1975; 93:1165–1168.
9. Madhuri D, Chandrasekhar KB, Somasekhar G, Harinadhababa K, Ramkotiah M, Dhandapani, B. Spectrophotometric determination of gatifloxacin through complexation with surfactant. *Int J Pharm Sci Res.* 2010; 1(2):84-89.
10. Naresh RAR. Kinetics and tissue distribution of niosomal bleomycin in tumor bearing mice. *Indian J Pharm Sci.* 1996; 58:230.
11. Baillie AJ, Florence AT, Hume LR, Muirhead GT, Rogerson A. The preparation and properties of niosomes non-ionic surfactant vesicles. *J Pharm Pharmacol.* 1985; 37,863–868.
12. Ruckmani KB. Jayakar, Ghosal SK. Nonionic surfactant vesicles (niosomes) of cytarabine hydrochloride for effective treatment of leukemia: encapsulation, storage and in vitro release. *Drug Dev Ind Pharm.* 2000; 26: 217–222.

13. Perrie Y, Barralet JE, McNeil S, Vangala A. A surfactant vesicle mediated delivery of DNA vaccines via the subcutaneous route. *Int J Pharm.* 2001; 284:31-41.
14. Kandasamy R, Veinramuthu S. Formulation and Optimization of Zidovudine Niosomes, *AAPS PharmSciTech*, 2010; 11(3), 1119-1127.
15. Leung SS, Robinson J R. The contribution of anionic polymer structural features to mucoadhesion. *J Controlled Release.* 1988; 5:.223-227.
16. Saitpi-Miranda V, Labret F, Grand-Boyer A, Gellis C, Chopineau J. Impact of deep freezing on the stability of vancomycin ophthalmic solutions. *Int J Pharm.* 2002; 234:205-207.
17. Tamilvanana S, Benitab, S. The potential of lipid emulsion for ocular delivery of lipophilic drugs. *Eur J Pharm Biopharm.* 2004; 58:357–368.
18. Colo DG , Burgalassi S , Chetoni P , Fiaschi MP. ‘Gel-forming erodible inserts for ocular controlled delivery of ofloxacin’ *Int J Pharm.* , 2001, 215:101–111.
19. Ghada Abdelbary and Nashwa El-gendy. Niosome-Encapsulated Gentamicin for Ophthalmic Controlled Delivery. *AAPS PharmSciTech*, 2008; 9(3),740-746.
20. Roser M, Fischer D, Kiser T. Surface-modified biodegradable albumin nano and microspheres. II: Effect of surface charges on in vitro phagocytes and biodistribution in rats. *Eur J Pharm Biopharm.* 1998; 46:255–63.

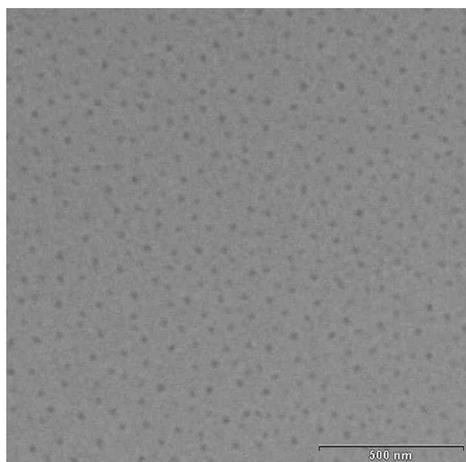


Fig 1 TEM image of niosome (F2)

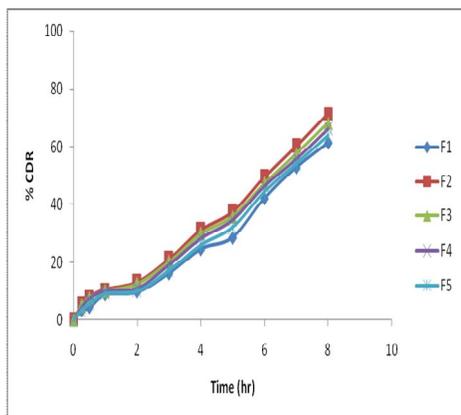


Fig.2 *In vitro* release of gatifloxacin from niosome formulations.

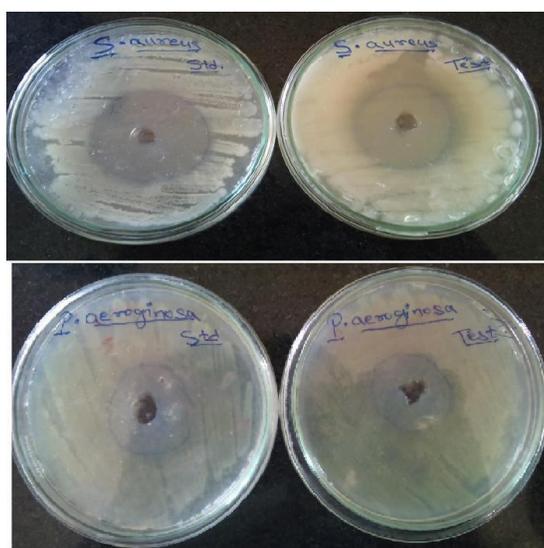


Fig 3 Antimicrobial efficacy testing of standard (Gatiquin[®] aqueous eye drop) and test (niosome formulation F2) against *S.aureus* and *Pseudomonas aeruginosa*

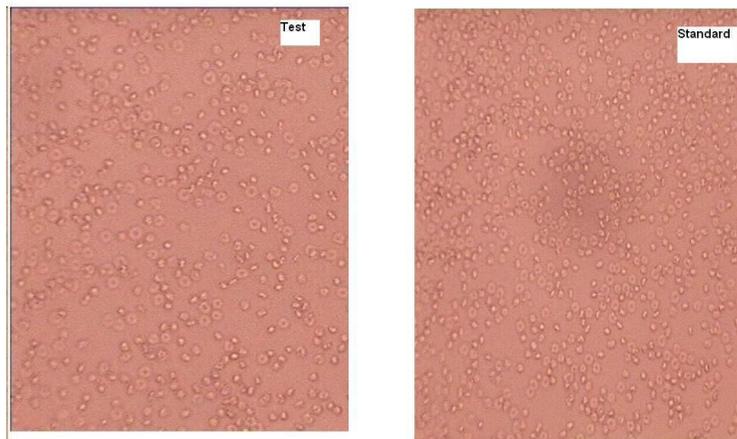


Fig 4. Photomicrograph of blood cells after mixing blood with test (niosome suspension) and standard (Gatiquin[®] aqueous eye drop).



Fig 5 Ocular irritancy testing using rabbits

Formula	Cholesterol	Span 60	Stearylamine (SA)
F1	0.5	1	0.1
F2	1	1	0.1
F3	1.5	1	0.1
F4	1	1.5	0.1
F5	1	0.5	0.1

Table-1 Formulae composition (Molar ratio)

Formulation	Entrapment Efficiency ^a (% ±SD)	Vesicle size (nm, average ±SD)	Zeta potential (mV)	Q ₈ ^b h (%)
F1	49.65 ± 1.20	719.00 ± 20.10	-21.4	61.33
F2	65.12 ± 1.79	883.00 ± 14.63	-30.1	71.45
F3	58.8 ± 2.19	1112.00 ± 19.16	-17.1	68.30
F4	53.2 ± 1.96	1147.00 ± 11.23	-31.9	66.18
F5	69.54 ± 1.63	1459 ± 11.25	-17.8	63.82

Table-2 Entrapment Efficiency, Vesicle Size, Zeta potential and Q_{8h} of gatifloxacin niosomes

^a Each value is an average of three determinations

^b Q_{8h}: % gatifloxacin released after 8 h.

Formulation	Percent Drug content on storage at $25\pm 2^{\circ}\text{C}$ and $75 \pm 5\% \text{RH}$			
	Initial	30 days	60 days	90 days
F2	100	92.23	91.72	92.04

Table-3 Stability study data