

# To Study the Formulation of Niosome of Ofloxacin and Its Evaluation for Efficacy of Anti-Microbial Activity

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**ABSTRACT:** Nanotechnology has offered enormous improvement in field of therapeutics by means of designing of nano-carriers that can cross biological barriers and are able to target cellular reservoirs. The aim of the present investigation was to formulate and evaluate Niosomes, a synthetic microscopic non-ionic surfactant vesicles formed on the admixture of a non-ionic surfactant, cholesterol and phosphate with subsequent hydration in aqueous media. The delivery of drugs by “vesicular drug delivery system” such as niosomes provides several important advantages over conventional drug therapy. 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 three 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. The Ofloxacin niosomes were prepared by lipid film hydration method. The prepared ofloxacin niosomes showed a vesicle size of 100-300 nm, the entrapment efficiency was 78.4%. The partition coefficient value of ofloxacin was found to be 0.5.

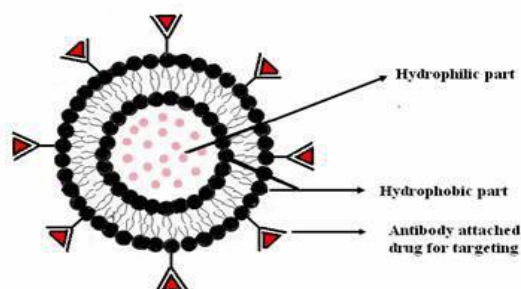
**KEYWORDS:** Ofloxacin, In-Vitro Release, Ether Injection Method, Entrapment Efficiency, Anti-Microbial Activity.

## I. INTRODUCTION

Niosomes are microscopic lamellar structures, which are formed on the admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. Structurally, niosomes are similar to liposomes, in that they are also made up of a bilayer. However, the bilayer in the case of niosomes is made up of non-ionic surface active agents rather than phospholipids as seen in case of liposomes.

A typical niosome vesicle would consist of a vesicle forming amphiphilic i.e. a non-ionic surfactant such as Span-60, which is usually stabilized by the addition of cholesterol and a small amount of anionic surfactant such as diacetyl phosphate, which helps in stabilizing the vesicle.

**Ofloxacin** is the fluoroquinolones are important antimicrobial agents that have demonstrated activity against a wide range of Gram-positive and Gram-negative organisms and have proved useful against micro-organisms that are resistant to other antibacterial agents.

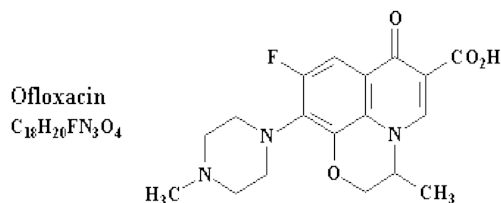


**Chemical Formula:**  $C_{18}H_{20}FN_3O_4$

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**Molecular Weight:** 361.4

**Indication:** For the treatment of infections such as respiratory tract, kidney, skin, soft tissue, urinary tract infection, urethral and cervical gonorrhoea.

**Melting Point:** Ofloxacin melts at 270 degree C to 273 degree C<sup>[1]</sup>

**Solubility:** Ofloxacin is freely soluble in PBS and acetic acid, slightly soluble in water, methanol, ethanol or acetone. The octanol/water partition coefficient for ofloxacin was reported to be -0.48 by<sup>[2]</sup>

**Appearance:** Ofloxacin occurs as a pale yellow or bright yellow crystalline powder, and with a bitter taste<sup>[3]</sup>

**Storage:** Ofloxacin should be stored at 40°C in the dark to minimise photolytically induced degradation<sup>[4]</sup>

**Pharmacology:** Ofloxacin is a quinolone antibiotic. Ofloxacin is bacterial and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to replicate one DNA double helix into two.

**Mechanism of Action:** Ofloxacin acts on DNA gyrase, an enzyme which, like human topoisomerase, prevents the excessive supercoiling of DNA during replication or transcription.

## II. RELATED WORK

Niosomes are biodegradable, biocompatible, nontoxic and capable of encapsulating large quantities of material in relatively smaller volumes of vesicles<sup>[5]</sup>

Drug delivery systems using colloidal particulate carriers such as liposomes or niosomes have distinct advantages over conventional dosage forms because the particles can act as drug containing reservoirs, and modification of the particle composition or surface can adjust the drug release rate and the affinity for the target site<sup>[6]</sup>

Proniosomes, hydrated by agitation in hot water for a short period of time, offer a versatile vesicle delivery concept with the potential for drug delivery via the transdermal route<sup>[7]</sup>

Temperature change of niosomal system affects assembly of surfactants into vesicles and also induces vesicle shape transformations<sup>[10]</sup>

Niosomes prepared from span 60 exhibits highest protection of insulin against proteolytic enzymes and good stability in presence of sodium deoxycholate and storage temperature. Ofloxacin is freely soluble in acetic acid, slightly soluble in water, methanol, ethanol or acetone. The octanol/water partition coefficient for ofloxacin was reported to be -0.48<sup>[1,2]</sup>

Ofloxacin which is the focus of this review is a second generation fluoroquinolone with a 6-fluoro substituent and a 7-piperazinyl substituent on the quinolone ring structure<sup>[11]</sup>

Entrapped niosomes were prepared by hand shaking and ether injection process with different ratios of (1:1, 1:2 and 1:3) cholesterol and Span-80 (Non-ionic surfactant). The niosomes prepared were in the size of 0.5-2.5 microns in case of hand shaking process and 0.5-2.5 microns in case of Ether injection process.

The release of drug from niosomes is determined using the membrane diffusion technique. Suspend an accurately measured amount of drug niosomal formulation in 1ml phosphate buffer saline and transferred to a glass tube covered at its lower end by a soaked cellulose membrane<sup>[12]</sup>

Drug delivery particle, an "immunoniosome" has been successfully synthesized and demonstrated the use of particle to target a specific site important in the inflammatory process, both in static cell culture and under flow<sup>[13]</sup>

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## III. MATERIALS AND METHODS

### MATERIALS REQUIRED

#### Chemicals:

- Span 60 (sorbitane monoesterate, CDH lab reagent), Cholesterol (LOBA CHEME), Ofloxacin drug (Zydus Cadila), Chloroform (Ramkem), Phosphate Buffer Saline (pH 7.4), 0.1 N Hydrochloric acid, 0.1 N Sodium Hydroxide, Sodium Sulfate, Sulfuric acid, 0.1 N Glacial Acetic acid, Ammonium Hydroxide, Sodium Chloride, Ethanol, Methanol, Acetone, Dialysis membrane (LA390-60), Nutrient Agar and Dialysis membrane were from Hi Media.

#### Instruments:

- Hot air oven (Scientech, 325 L), Incubator (Scientech), Digital Balance (Denver, Germany), Autoclave-vertical, Laminar air flow chamber horizontal (Scientech), Shimadzu 1800 Double Beam UV-VIS Spectrophotometer (Japan), Centrifuge (Remi C-24 India), Vortex Mixer (Remi), Magnetic stirrer with hot plate (Remi), Digital pH meter (Eutech, Singapore), Vacuum Rotary Evaporator (Buchi Type), Trinocular Microscope (Olympus CH 20 I) with Ocular Micrometer.

### METHODOLOGY

#### Quantitative Estimation of Ofloxacin:

Spectrophotometric method was done as reported<sup>[5]</sup>. Ofloxacin was prepared in PBS in concentration range of 10 mg in 100 ml. Different aliquots in gradation of 0.2, 0.4.....2 ml volume were taken in each test tube and the final volume was made to 10 ml with PBS. The absorbance was taken at 287 nm and standard plot were prepared by Shimadzu 1800 U.V. Visible Spectrophotometer.

**Preformulation Study:** Preformulation study is the prime requisite which is required to be done before using any drug for the preparation of dosage form. Before the preparation of noisome the following preformulation studies were done for ofloxacin.

- Physical Appearance:** Ofloxacin sample which was obtained from Zydus Cadila Ahmedabad was minutely observed for the physical appearance. The drug used was whitish yellow, amorphous and odourless powder.
- Melting Point:** Ofloxacin study was conducted to test melting point and it was found that ofloxacin melt within the range of 270°C -273°C.
- Solubility Profile:** Solubility is defined as spontaneous interaction of two or more substances to form a homogeneous molecular dispersion. The solubility of ofloxacin is tested in various solvents.

SL. No.	Solvent	Solubilty
01.	Methanol	Freely Soluble
02.	Ethanol	Sparingly Soluble
03.	Distill Water	Slightly Soluble
04.	Acetone	Soluble
05.	Saline	Soluble
06.	PBS (pH 7.4)	Soluble
07.	0.1 N HCL	Soluble
08.	0.1 N NaOH	Slightly Insoluble

- Partition Coefficient:** Partition coefficient is defined as the ratio of unionized drug distributed between organic and aqueous phase at equilibrium.

$$P_{o/w} = \frac{C_{oil}}{C_{water}}$$

The partition coefficient of ofloxacin was determined in n-octanol : PBS and was found to be 0.5.

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### Selection of Surfactant:

Surfactants are backbone of the preparation of basic composition of the niosome. During the present study the non-ionic surfactant of sorbitol ester class were selected. From this class Span- 40 and Span - 60 were chosen for the preparation of niosome. These surfactants were used in the following ratios:

1. Surfactant : Cholesterol 1:1
2. Surfactant : Cholesterol 1:2
3. Surfactant : Cholesterol 2:1

### Formulation of Niosome:

SL.No.	Formulation Code	Surfactant Span 60	Cholesterol	Solvent	Drug in Aqueous Solution
01.	A1	50 mg	50 mg	10 ml of Diethyl Ether	200 mg of Ofloxacin + 10 ml of PBS
02.	A2	50 mg	100 mg	10 ml of Diethyl Ether	200 mg of Ofloxacin + 10 ml PBS
03.	A3	100 mg	50 mg	10 ml of Diethyl Ether	200 mg of Ofloxacin + 10 ml of PBS

### Preparation of Niosomes:

In the present study the niosome were prepared using ether injection method and hand shaking method (Thin Film Hydration) and the comparative study were done between niosome prepared by above two methods.

#### 1. Ether Injection Method:

10 ml of Diethyl Ether was taken in 100 ml beaker. To it surfactant-cholesterol mixture was added in appropriate ratio. This solution was filled in a 20 ml syringe of 40 gauge. In a 250 ml beaker 10 ml PBS was taken. To it 200 mg of Ofloxacin was added. The beaker was kept in magnetic stirrer in heating condition. The contents of the syringe were injected drop wise into a mixture. The procedure was repeated six times with various samples A1, A2, A3, B1, B2 and B3.

#### 2. Hand Shaking Method (Film Hydration):

The mixture of vesicle forming ingredients ie. Surfactant and Cholesterol was dissolved in a volatile organic solvent. 10 ml Diethyl Ether was taken in a round bottom flask. The organic solvent was removed at room temperature (37°C) using Rotary Vacuum Evaporator- Buchi type leaving a thin layer of solid mixture. The dried film of surfactant and cholesterol was rehydrated with 200 mg of Ofloxacin contained in 1ml of PBS with gentle agitation. The niosomes were carefully removed and kept aside for further analysis.

### Evaluation of Niosomes:

#### ➤ Determination of Vesicle Shape and Size by Microscopy:

For determining the vesicle shape and size freshly prepared samples of niosome were examined under Olympus optical microscope. The size of the niosome was measured by using precalibrated micrometer. The least count of ocular micrometer was calculated as 1.53 μm and around 10 particles were analyzed in each of the samples.

### Determination of Vesicle Size of Niosome Composed of Span 60

#### A1 Formulation:

Sl. No.	No. of Division	Diameter (μm)	No. of Division	Diameter (μm)
01.	3	4.59	12	18.36
02.	2	3.06	7	10.71
03.	4	6.12	13	19.89
04.	9	13.77	8	12.24

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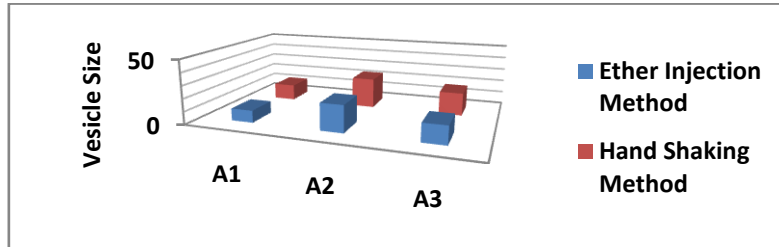
05.	4	6.12	6	9.18
06.	8	12.24	11	16.83
07.	6	9.18	6	9.18
08.	11	16.83	4	6.12
09.	5	7.65	5	7.65
10.	10	15.3	11	16.83
	<b>Average Diameter</b>	<b>9.486</b>	<b>Average Diameter</b>	<b>12.699</b>

**A2 Formulation:**

SL. No.	No. of Division	Diameter (µm)	No. of Division	Diameter (µm)
01.	8	12.24	11	16.83
02.	15	22.95	25	38.25
03.	9	13.77	23	35.19
04.	11	16.83	12	18.36
05.	15	22.95	19	29.07
06.	17	26.01	17	26.01
07.	10	15.3	15	15.3
08.	21	32.13	11	16.83
09.	23	35.19	17	26.01
10.	9	13.77	10	15.3
	<b>Average Diameter</b>	<b>21.11</b>	<b>Average Diameter</b>	<b>23.715</b>

**A3 Formulation:**

SL.No.	No. of Division	Diameter (µm)	No. of Division	Diameter (µm)
01.	7	10.71	11	16.83
02.	9	13.89	8	12.24
03.	10	15.3	13	19.89
04.	9	13.77	17	26.01
05.	10	15.3	8	12.24
06.	6	9.18	12	18.36
07.	11	19.83	11	19.83
08.	15	22.95	13	19.89
09.	6	9.18	10	15.3
10.	8	12.24	9	19.89
	<b>Average Diameter</b>	<b>14.23</b>	<b>Average Diameter</b>	<b>18.048</b>



**Fig: Effect of Method of Preparation on the Vesicle Size of Niosome Prepared by using Span 60**

➤ **Determination of Entrapment Efficiency:**

The niosomal preparations which were prepared using different concentration of cholesterol and surfactants were taken. All of the preparations were centrifuged in REMI table top centrifuge to separate the niosomal vesicle as a pellet. The supernatant was collected and analyzed for ofloxacin content. The amount of drug un entrapped was calculated which finally gave the % entrapment efficiency.

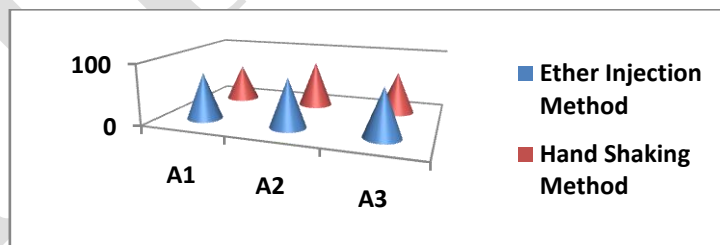
$$\text{Entrapment Efficiency} = \frac{\text{Amount of Ofloxacin Entrapped}}{\text{Total Amount of Drug}} \times 100$$

**Hand Shaking Method :**

SL. No.	Sample Code	Amount of Drug in Supernatant	Amount of Drug Entrapped in Niosome	% Entrapment Efficiency
01.	A1	45.62	154.38	77.19
02.	A2	39.67	160.33	80.16
03.	A3	43.839	156.17	78.08

**Ether Injection Method :**

SL. No.	Sample Code	Amount of Drug in Supernatant	Amount of Drug Entrapped in Niosome	% Entrapment Efficiency
01.	A1	76.32	123.68	61.84
02.	A2	44.77	155.23	77.61
03.	A3	61.17	138.83	69.41



**Fig: Effect of Method of Preparation on the Entrapment Efficiency of Niosome Prepared by using Span 60**

**Determination of Invitro Release Profile of Ofloxacin:**

The niosomal preparations which were separated in the form of a pellet were collected carefully in test tubes. The pellet was suspended in a 5 ml of PBS. The sample was taken dialysis tube. The aliquots were withdrawn and analyzed for ofloxacin release. The percentage release was then calculated in different time interval was calculated.

Time (hrs.)	A1	A2	A3
0	0.000	0.000	0.000
1	5.920	6.870	8.020
2	14.230	14.270	14.330
3	18.930	18.980	19.040
4	25.550	24.840	25.850
5	29.980	30.940	32.190
6	37.240	37.680	38.610
7	40.980	43.730	44.310
8	44.060	47.520	48.310
9	47.600	51.980	52.220
10	51.590	55.980	55.660
11	67.910	70.580	72.330

Table: Effect of surfactant (Span 60) on in vitro release profile of Ofloxacin in PBS at 37±2 °C.

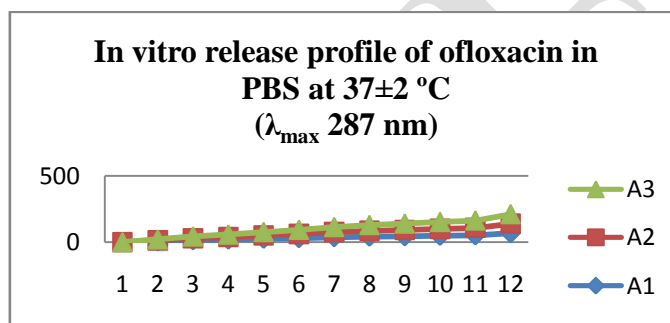


Fig: In Vitro Release Profile of Ofloxacin in PBS

**In Vitro Anti-Bacterial Studies:**

In vitro anti-bacterial inhibitory activity of ofloxacin from niosomal formulations were conducted using disc diffusion method and *Escherichia coli* as a test organism. Inhibition zone values for niosomes formulations were measured individually.

SL. No.	Formulation Code	Plate- 1	Plate- 2	Plate- 3
01.	A1	12	14	11
02.	A2	21	22	19
03.	A3	10	11	9

It was found that values for A2 formula were significantly more from other respective formulations. It was observed from above mentioned results that formula A2 had highest zone of inhibition. Hence, it may be concluded that formulation A2 exhibited better antimicrobial activity in comparison to respective formulation.

**IV. RESULTS AND DISCUSSION**

In the first step, Ofloxacin niosomes were prepared by lipid film hydration method in which mixture of surfactant, cholesterol and drug was taken and dissolved in organic solvents. The organic solvents were removed by rotary evaporation method, so as to form a thin film of components on the wall of the rotating flask. The film was hydrated with 8-10 ml of PBS for 45 min at room temperature with gentle shaking the niosomal suspension was further hydrated for 24 hrs. Niosomes were also prepared by Ether Injection method.

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The effect of varying cholesterol ratio has direct effect on the integrity of vesicle membrane. Entrapment efficiency increases with increasing cholesterol concentration but only up to a certain limit this may be because the cholesterol has the ability to cement the leaking space in bilayer membranes, but after a definite concentration it results in leaky vesicles.

In vitro release of ofloxacin from niosome systems were estimated. It was concluded that the release behaviour of A2 formulation of Span 60 was better.

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#### REFERENCES

1. Ross DL, Riley CM. Aqueous solubilities of some variously substituted quinolone antimicrobials. *Int. J. Pharm.* 63: 237-250. 1990
2. Hiral K, Aoyanama H, Irikura T, Iyobe S, Mitsuhashi S. Differences in susceptibility to quinolones of outer membrane mutants of *Salmonella typhimurium* and *Escherichia coli*. *Antimicrob. Agents Chemother.* 29:535-538. 1986.
3. Ross DL, Elkinton S, Riley CM. Physicochemical Properties of the Fluoroquinolone Antimicrobials IV 1-Octanol/ Water Partition 379-389.1992.
4. Macias B, Villa MV, Sastre A, Castineiras A, Borrás J. Complexes of Co(II) and Zn(II) with ofloxacin crystal structure of [Co(II)(oflo)2(MeOH)2]4MeOH *Pharm. Sci.* 91 (11):2416-2423. 2002.
5. Park HR, Chung HC, Lee JK, Bark KM. Ionization and divalent cation complexation of Quinolones Antibiotics in aqueous solution. *Bull. Korean Chem. Soc.* 21(9) 849-854.2000.
6. Malhotra, M and Jain N.K. Niosome as drug carriers. *India Drugs*; 31(3):81-86.2004.
7. Betageri, G., Habib, M. Liposomes as drug carriers. *Pharm. Eng.* 14, 76-77.1994.
8. Schreier, H., Bouwstra, J. Liposomes and niosomes as typical drug carriers: dermal and transdermal drug delivery. *J. Controlled Release* 30, 1-15.1994.
9. Blazek- Welsh A, Rhodes DG. SEM, Maltodextrin- based proniosomes AAPS pharma sci (serial online), 3 1.2004.
10. Arunothayanum P et al. Extrusion of niosomes from capillaries approaches to a pulsed delivery device. *J. Cont. Release* 60:391. 1999.
11. Sato K, Matsuura Y, Inoue M, Une T, Osada Y, Ogawa H, Mitsuhashi S. In- vitro and in-vivo activity of DL-8280 (or-ofloxacin), a new oxazine. *Antimicrob. Agents Chemother.*, 23:548-553, 1982.
12. Ahmed S, Guinedi, Nahed D, Mortada, Samar Mansour, Rania M. Hathout. Preparation and evaluation of reverse phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide. *Int. J. Pharm.* 306, 71-82.2005.
13. Vora B, Khopade AJ, Jain NK. Proniosomes based transdermal delivery of Levonorgestrel for effective contraception, *J.Control. Rel.* 54, 149-165.1998.
14. Chandraprakash K.S., Udupa N., Umadevi P. And Pillai G.K. Pharmacokinetic evaluation of surfactant vesicles containing methotrexate in tumour bearing mice. *Int. J. Pharma.*; R1-R3: 61.1990
15. Chauhan S. And Luorence M.J. The preparation of polyoxyethylene containing non-ionic surfactant. Vesicles. *J.Pharm.Pharmacol.* 41:6p. 1998.
16. Gayatri Devi S., Venkatesh P. And Udupa N. Niosomal sumatriptan succinate for nasal administration. *Int.J. Pharm. Sci.*; 62(6), 479-481.2000
17. Hood, E, Gonzalez, M, Plaas, A, Strom, J and Vanauker, M. Immuno-targation of non-ionic surfactant vesicles to inflammation. *International Journal of Pharmaceutics*, 339,222. 2007.
18. Suzuki K. And Sokan K. The Application of Liposomes to Cosmetics. *Cosmetic and Toiletries.* 105:65-78. 1990.
19. Yoshida H., Lehr C.M., Kok W., Junginger H.E., Verhoef J.C. and Bouwistra J.A. Niosomes for oral delivery of peptide drugs. *J.Control Rel.*; 21:145-153. 1992.
20. Yoshioka T., Sternberg B., Moody M. And Florence A.T. Niosomes from Span surfactants: Relations between structure and form. *J. Pharm. Pharmacol. Supp.* 44-1044.1992.