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Formulation and evaluation of levofloxacin niosomes

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ABSTRACT

The delivery of drugs by "vesicular drug delivery system" such as nano-niosomes provides several important advantages over conventional drug therapy. This study reports the development of a highly stable niosomal nanostructure based on Span 60, span 80/cholesterol, chitosan system and its potential application for oral delivery of Levofloxacin. Levofloxacin loaded niosomes were prepared by reversed-phase evaporation and Chitosan coating was performed by incubation of niosomal suspensions with Chitosan solution. The prepared niosomes were characterized for entrapment efficiency (EE), *in vitro* drug release, drug release kinetics, particle size, zeta potential, surface morphology, anti microbial activity and stability study. Highest entrapment efficiency was observed in LNF-3 79.11. The study of drug release kinetics showed that formulations The formulation LNF-3 released 99.03% of drug for the prolonged period of time (12 hours) than other formulations. governed by higuchi kinetic model (R^2=9792). Particle size and zeta potential of the LNF-3 formulation was found to be 339.40 nm with unimodal distribution (PDI 0.160), +23.1mV with spherical morphology. The *in-vitro a*ntimicrobial efficiency of optimized noisome formulation enhanced 2-fold by compared with Levofloxacin alone, revealed that niosomes formulations have stronger inhibitory activity. The optimized noisome formulation showed excellent stability in for over 90 days at 40°C.

Keywords: Levofloxacin, Niosomes, Reversed-phase evaporation, In-vitro study

INTRODUCTION

For many decades, medication of an acute disease or a chronic illness has been accomplished by delivering drugs to the patients via various pharmaceutical dosage forms like tablets, capsules, pills, creams, ointments, liquids, aerosols, injectable and suppositories as carriers. To achieve and then to maintain the concentration of drug administered within the therapeutically effective range needed for medication, it is often necessary to take this type of drug delivery systems several times in a day. This results in a fluctuated drug level and consequently undesirable toxicity and poor efficiency. To minimize this fluctuation, novel drug delivery systems have been developed, which include niosomes, liposomes, nano particles, microspheres, micro emulsions, impala table pumps and magnetic microcapsule **(**Allen 1998).

The concept of targeted drug delivery is designed for attempting to concentrate the drug in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues. As a result, drug is localised on the targeted site. Hence, surrounding tissues are not affected by the drug. In addition, loss of drug does not happen due to localisation of drug, leading to get maximum efficacy of the medication. Different carriers have been used for targeting of drug, such as immunoglobulin, serum proteins, synthetic polymers, liposome, microspheres, erythrocytes and Niosomes (Chen X 2012).

Niosomes are one of the best among these carriers. Structurally, niosomes are similar to liposomes and also are equiactive in drug delivery potential but high chemical stability and economy makes niosomes superior than liposomes. Both consist of bi layer, which is made up of non-ionic surfactant in the case of niosomes and phospholipids in case of liposomes. Niosomes are microscopic lamellar structures of size range between 10 to1000 nm and consists of biodegradable, non-immunogenic and biocompatible surfactsnts4. The niosomes are amphiphilic in nature, which allows entrapment of hydrophilic drug in the core cavity and hydrophobic drugs in the non-polar region present within the bilayer hence both hydrophilic and hydrophobic drugs can be incorporated into niosomes.

MATERIALS & METHODS

Materials

levofloxacin(BP/USP) was the active pharmaceutical ingredient obtained yarrow chemicals, span 60, span 80, cholesterol, chitosan, diethyl ether were used a excipients to design levofloxacin niosomes.

Method

Levofloxacin niosomes (LN) are prepared by reverse phase evaporation method. A mixture of span 60 and cholesterol (CH) (1:0.25, 1:0.5, 1:0.75, 1:1, 1:1.25 molar ratio) was dissolved in 20 ml of diethyl ether, in a flask. Subsequently, 200mg levofloxacin solution in a 3 ml phosphate buffer (pH 6.8) was added to the flask and mixed for 1min. The mixture was then emulsified using 10,15 and 20 min sonication in a water bath at 10ºC. The mixture was rotary evaporated at 40ºC with a rotating speed 50 rpm for about 15min to remove the organic solvent. The suspension finally underwent high pressure extrusion, while passing two filters of different sizes (0.22, 0.45µm). The suspension was passed from these filters for five times. Free levofloxacin was removed by two step ultra centrifuge (10000 rpm, 4ºC, and 10 min) and washed with phosphate buffer. Chitosan (CS) was carried out by incubation of the niosomal suspensions with the chitosan solution (2mg/ml) at 37ºC for at least 1 h. Free chitosan was finally removed from the suspension by two step Ultra centrifugation (10000 rpm, 4ºC, and 10min) and then washed with phosphate buffer.

Table No.1: Composition of Levofloxacin niosomes formulations

INGREDIENTS	LNF 1	LNF 2	LNF 3	LNF 4	LNF 5	LNF 6	LNF 7	LNF 8	LNF 9	LNF 10
Levofloxacin(mg)	200	200	200	200	200	200	200	200	200	200
Span 60(mg)	100	100	100	100	100	-	-	-	-	-
Span 80(mg)	-	-	-	-	-	100	100	100	100	100
Cholesterol (mg)	25	50	75	100	125	25	50	75	100	125
Chitosan(mg)	2	2	2	2	2	2	2	2	2	2
Diethyl ether (ml)	20	20	20	20	20	20	20	20	20	20

EVALUATION TESTS

Generally evaluation test performed for the niosomes listed the followed entrapment efficiency, compatibility studies, *in vitro* drug release, particle size, zeta potential, microscopic analysis, anti microbial activity.

Entrapment Efficiency

Table No.2: Entrapment efficiency of different formulations

S.No	Formulation code	Entrapment Efficiency (%)
1	LNF1	52.12 ±4.15
2	LNF2	64.33 ±2.22
3	LNF3	79.11± 3.67
4	LNF4	82.20 ±2.35
5	LNF5	80.11± 3.45
6	LNF6	49.13± 2.75
7	LNF7	55.62± 3.54
8	LNF8	62. 73±4.43
9	LNF9	71.34 ± 3.43
10	LNF10	76.20± 4.44

Results expressed in Mean ± SD (n=3)

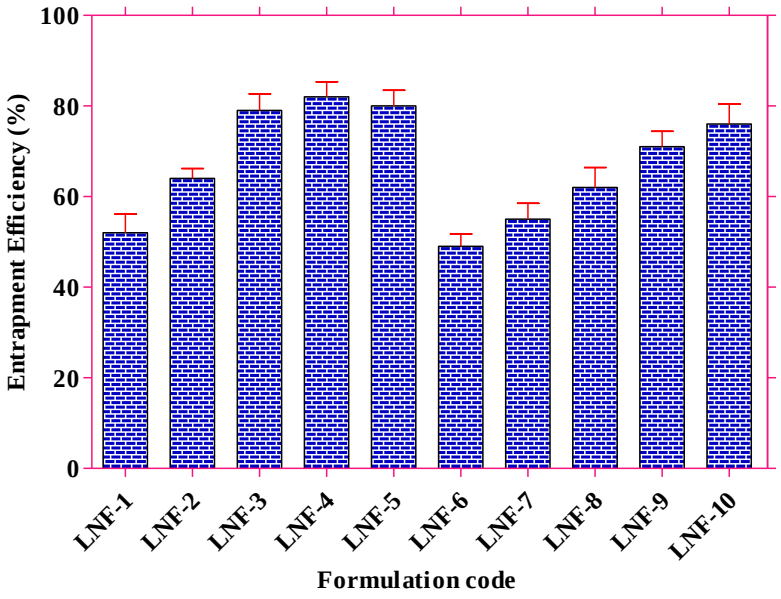


Figure No.1: Entrapment efficiency of different formulations

Drug-Excipients compatibility study

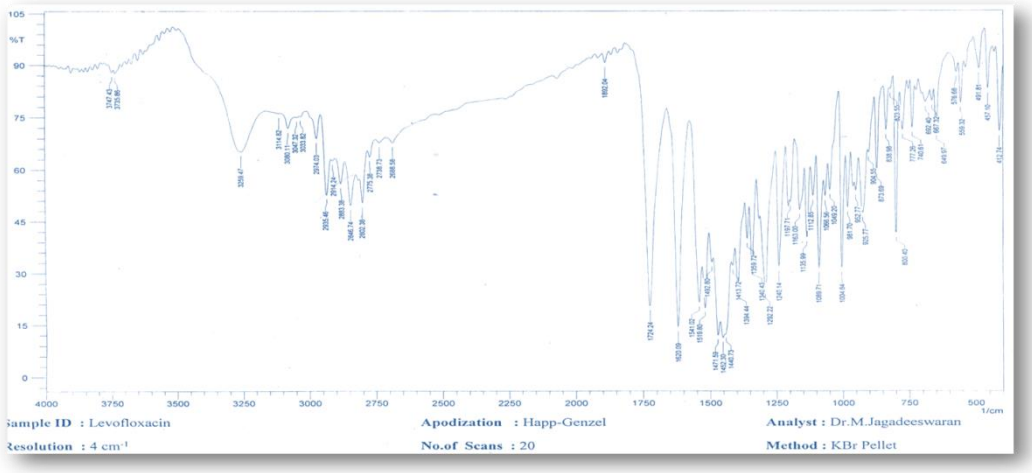


Figure NO.2: FTIR Spectra of levofloxacin

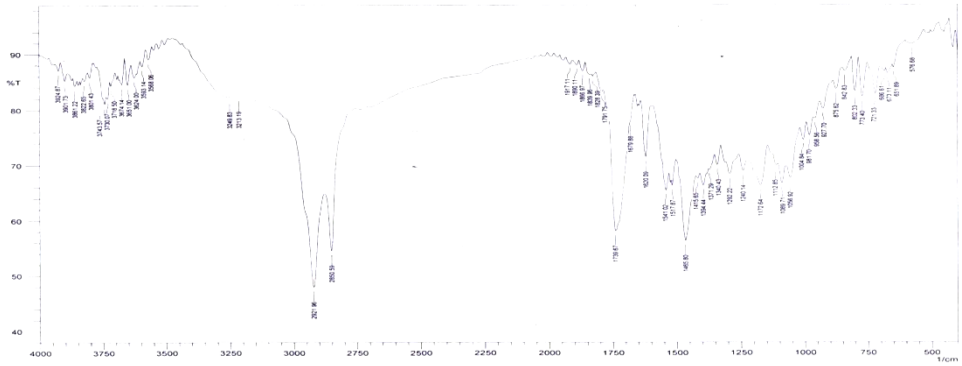


Figure No.3: FTIR Spectra of Optimized formulation (LNF-3)

In vitro drug release studies

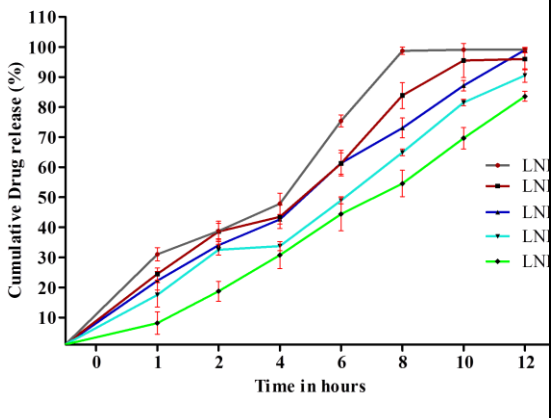


Figure 4: *In vitro* drug release profile of LNF-1-LNF-5

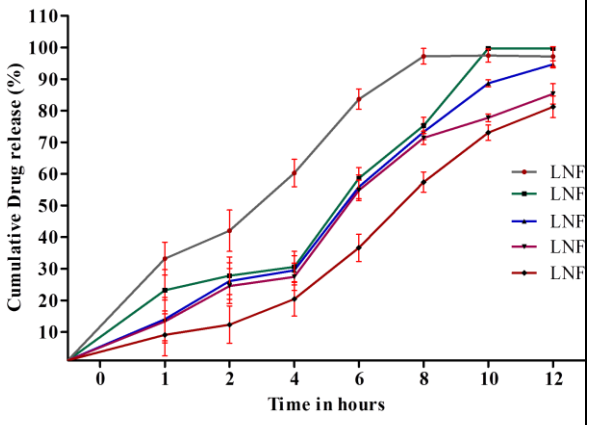
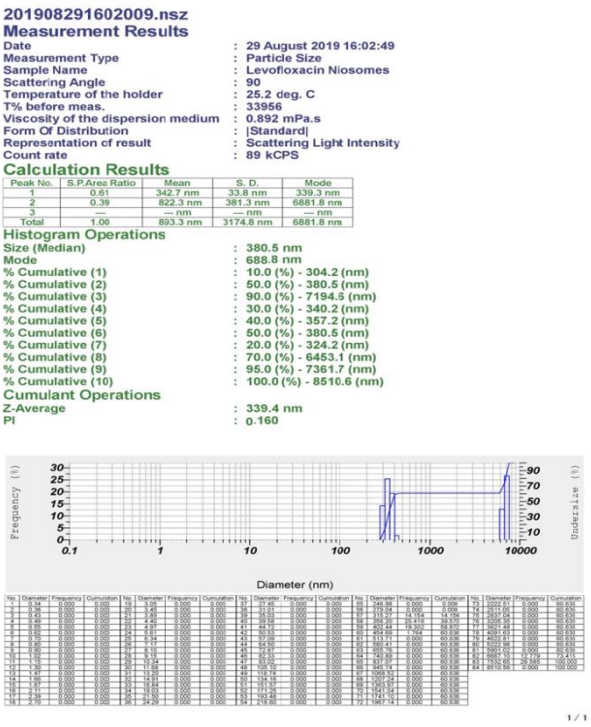


Figure 5: *In vitro* drug release profile of LNF-6-LNF-10



201908291602009.nsz
Measurement Results

Date	: 29 August 2019 16:02:49
Measurement Type	: Particle Size
Sample Name	: Levofloxacin Niosomes
Scattering Angle	: 90
Temperature of the holder	: 25.2 deg. C
T% before meas.	: 33956
Viscosity of the dispersion medium	: 0.892 mPa.s
Form Of Distribution	: |Standard|
Representation of result	: Scattering Light Intensity
Count rate	: 89 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.61	342.7 nm	33.8 nm	339.3 nm
2	0.39	822.3 nm	381.3 nm	6881.8 nm
3	---	--- nm	--- nm	--- nm
Total	1.00	893.3 nm	3174.8 nm	6881.8 nm

Histogram Operations

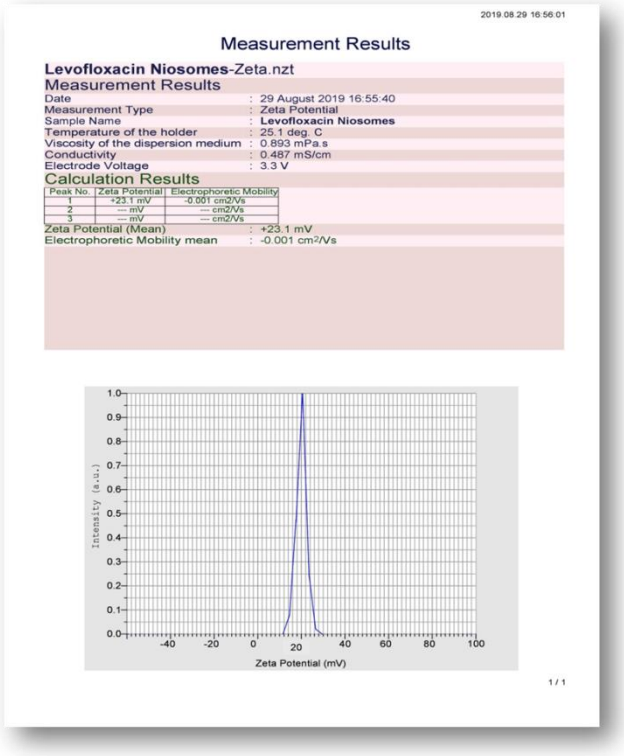
Size (Median)	: 380.5 nm
Mode	: 688.8 nm
% Cumulative (1)	: 10.0 (%) - 304.2 (nm)
% Cumulative (2)	: 50.0 (%) - 380.5 (nm)
% Cumulative (3)	: 90.0 (%) - 7194.6 (nm)
% Cumulative (4)	: 30.0 (%) - 340.2 (nm)
% Cumulative (5)	: 40.0 (%) - 357.2 (nm)
% Cumulative (6)	: 50.0 (%) - 380.5 (nm)
% Cumulative (7)	: 20.0 (%) - 324.2 (nm)
% Cumulative (8)	: 70.0 (%) - 6453.1 (nm)
% Cumulative (9)	: 95.0 (%) - 7361.7 (nm)
% Cumulative (10)	: 100.0 (%) - 8510.6 (nm)

Cumulant Operations

Z-Average	: 339.4 nm
PI	: 0.160

Figure No.6: Vesicle size of LNF-3

Particle surface charge (zeta potential)



2019.08.29 16:56:01

Measurement Results

Levofloxacin Niosomes-Zeta.nzt
Measurement Results

Date	: 29 August 2019 16:55:40
Measurement Type	: Zeta Potential
Sample Name	: **Levofloxacin Niosomes**
Temperature of the holder	: 25.1 deg. C
Viscosity of the dispersion medium	: 0.893 mPa.s
Conductivity	: 0.487 mS/cm
Electrode Voltage	: 3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	+23.1 mV	-0.001 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	: +23.1 mV
Electrophoretic Mobility mean	: -0.001 cm²/Vs

1 / 1

Figure No.7: Zeta potential of LNF-3

Microscopic analysis of selected niosomes formulation

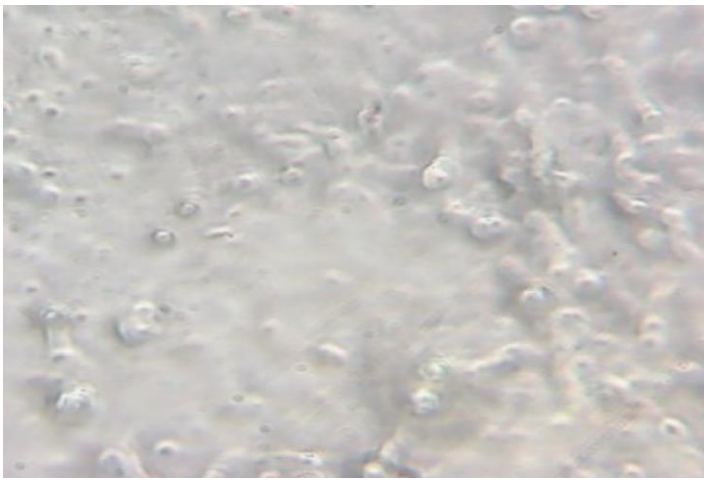


Figure No.8: Optical photomicrograph of LNF-3 at (X400)

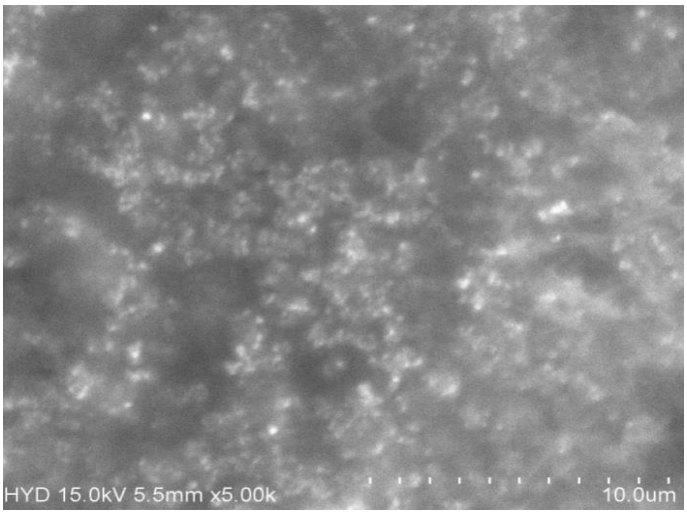


Figure No.9: Scanning electron micrograph LNF-3 at (X5000)

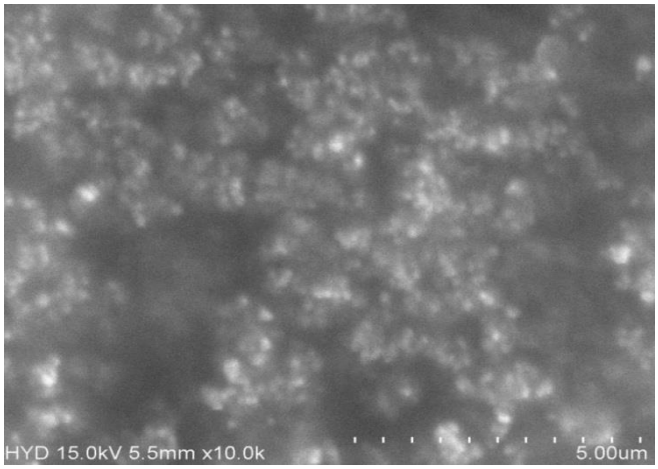
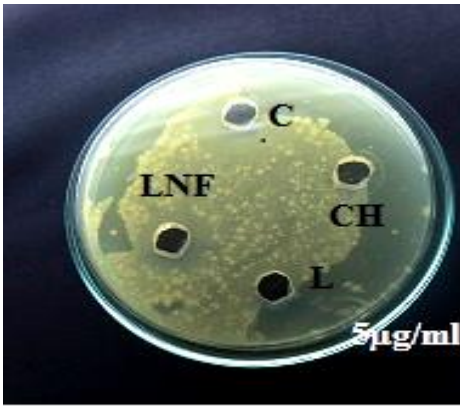


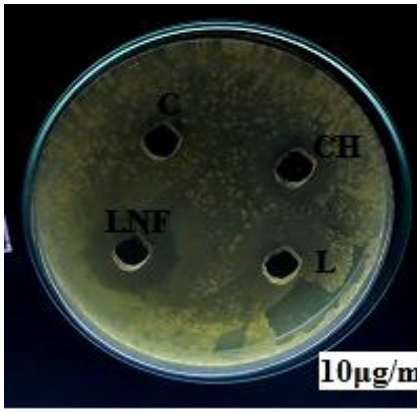
Figure No.10: Scanning electron micrograph LNF-3 at (X10000)

ANTI MICROBIAL ACTIVITY



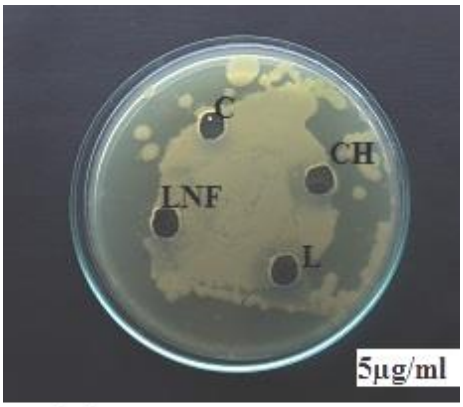
Bacillus subtilis

(a) C: control, CH :Chitosan 5µg/ml, LNF-3: Levofloxacin niosomes µg/ml, L : Levofloxacin: 5µg/ml



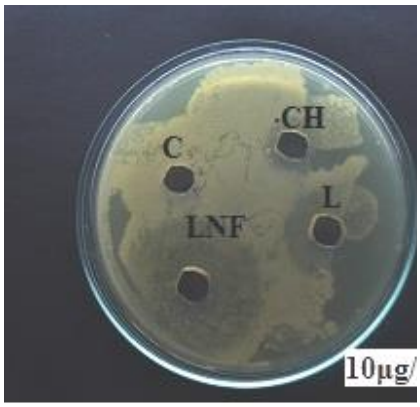
Bacillus subtilis

(b) c: control, CH: Chitosan: 10µg/ml, LNF-3: Levofloxacin niosomes10 µg/ml, L: Levofloxacin:10 µg/ml



staphylococcus aureus

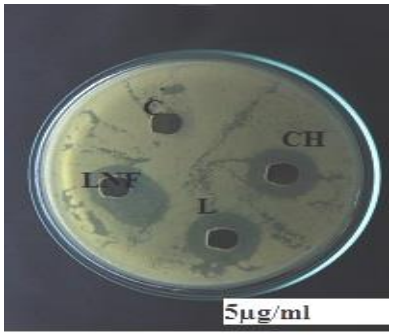
(c) c: control, CH :Chitosan 5µg/ml, LNF-3: Levofloxacin niosomes5µg/ml, L : Levofloxacin: 5µg/ml



Staphylococus aureus

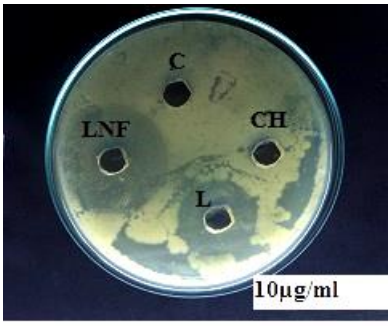
(d) c: control, CH: Chitosan: 10µg/ml, LNF-3: Levofloxacin niosomes: 10 µg/ml, L: Levofloxacin:10 µg/ml

Figure No.11: a and b Agar well diffusion assay of *Bacillus subtilis,* c and d Agar well diffusion assay of *Staphylococcus aureus*



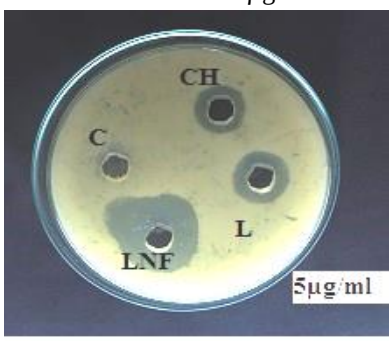
Escherichia Coli

a) c: control, CH: Chitosan 5µg/ml, LNF-3: Levofloxacin niosomes: 5µg/ml, L : Levofloxacin 5µg/ml



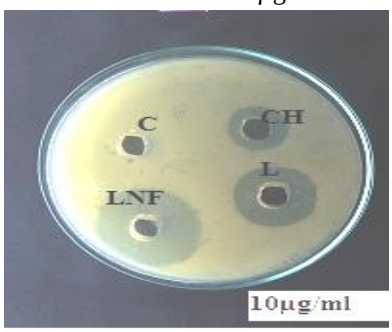
Escherichia Coli

(b) c: control, CH: Chitosan 10µg/ml, LNF-3: Levofloxacin niosomes: 10 µg/ml, L : Levofloxacin 10 µg/ml



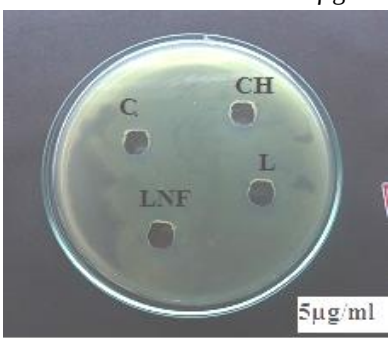
Klebsiella pneumoniae

(c) c: control, CH: Chitosan 5µg/ml, LNF-3: Levofloxacin niosomes: 5µg/ml, L : Levofloxacin 5µg/ml



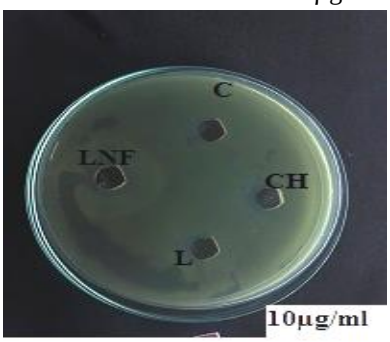
Klebsiella Pneumoniae

(d) c: control, CH: Chitosan 10µg/ml, LNF-3: Levofloxacin niosomes: 10 µg/ml, L : Levofloxacin 10 µg/ml



Salmonella Typhi

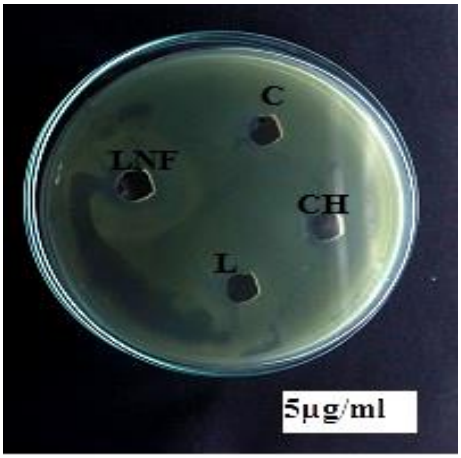
(e) c: control, CH: Chitosan 5µg/ml, LNF-3: Levofloxacin niosomes: 5µg/ml, L : Levofloxacin 5µg/ml



Salmonella Typhi

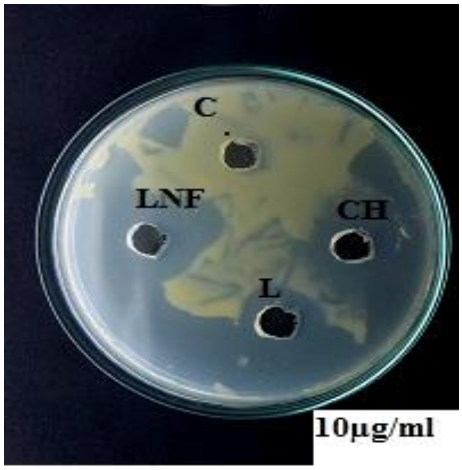
(f) c: control, CH: Chitosan 10µg/ml, LNF-3: Levofloxacin niosomes: 5µg/ml, L : Levofloxacin 5µg/ml

Figure No. 12: a and b Agar well diffusion assay of *Escheschia coli*, c and d Agar well diffusion assay of *Klebsiella pneumonia*, e and f Agar well diffusion assay of *Salmonella typhi*



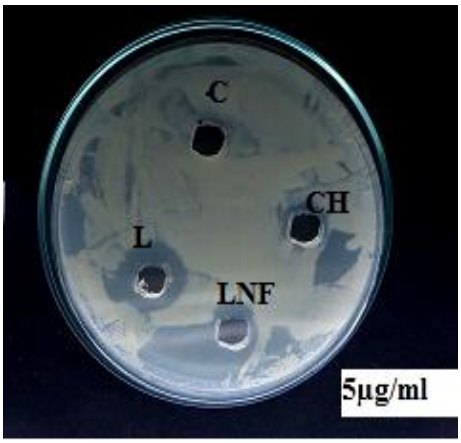
Candida albicans

(a) c: control, CH: Chitosan 5µg/ml, LNF-3:Levofloxacin niosomes 5µg/ml, L : Levofloxacin 5µg/ml



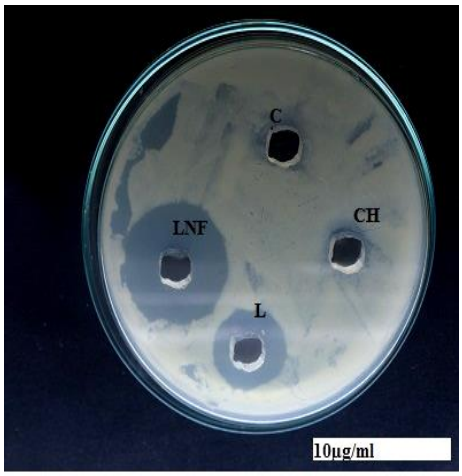
Candida albicans

(b) c: control, CH: Chitosan 10µg/ml, LNF-3: Levofloxacin niosomes: 10 µg/ml, L : Levofloxacin 10 µg/ml



Aspergillus niger

(c) c: control, CH: Chitosan 5µg/ml, LNF-3: Levofloxacin niosomes 5µg/ml, L : Levofloxacin 5µg/ml

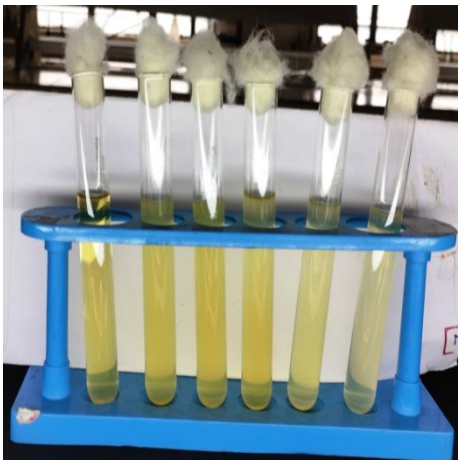


Aspergillus niger

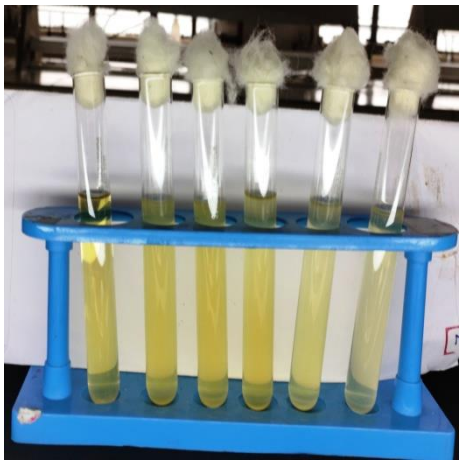
(d) c: control, CH: Chitosan 10µg/ml, LNF-3: Levofloxacin niosomes, 10 µg/ml, L : Levofloxacin 10 µg/ml

Figure No.13: a and b Agar well diffusion assay of *Candida albicans* , c and d Agar well diffusion assay of *Aspergillus niger*

Minimal Inhibitory Concentration

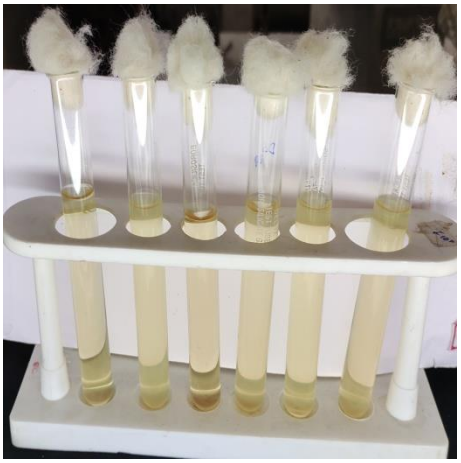


(a) *Bacillus subtillis* +

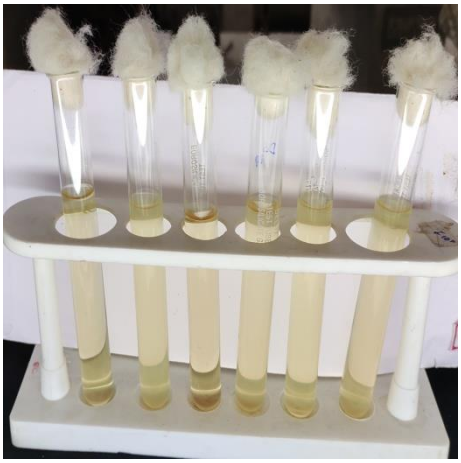


(b) *Staphylococcus aureus*+ levofloxacin

Levofloxacin



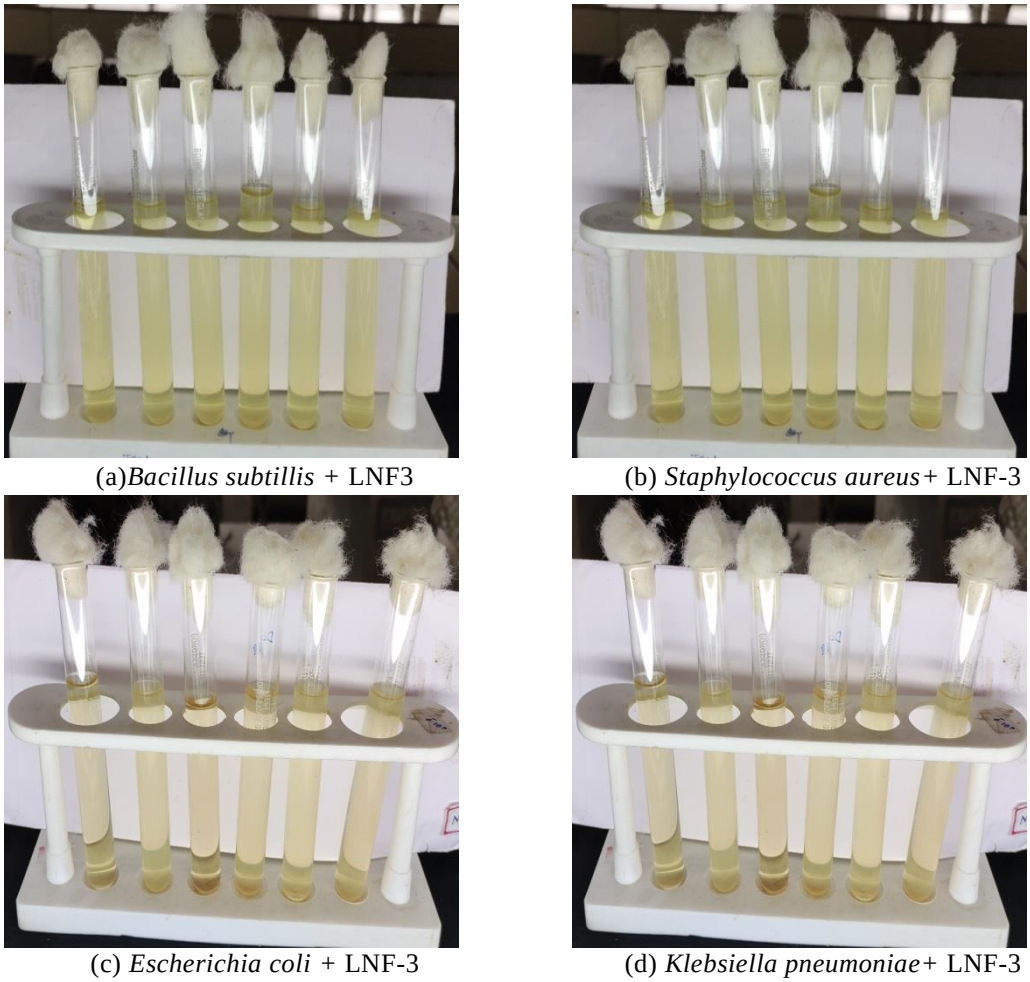
(c) *Escherichia coli* +



(d) *Klebsiella pneumoniae*+ levofloxacin

Levofloxacin

Figure No.14: Minimal inhibitory concentration (MIC) of levofloxacin against various microorganisms by tube dilution method



(a)*Bacillus subtillis* + LNF3 (b) *Staphylococcus aureus*+ LNF-3

(c) *Escherichia coli* + LNF-3 (d) *Klebsiella pneumoniae*+ LNF-3

Figure No.15: Minimal inhibitory concentration (MIC) of LNF-3 against various microorganism by tube dilution method.

PREFORMULATION STUDIES IDENTIFICATION OF DRUG

The IR spectrum of pure drug was found to be similar to the reference standard IR spectrum of levofloxacin.

Table 3: FTIR data for estimation of levofloxacin

S. No	Functional groups	Wave number(cm^{-1})
1	N-CH_3	2825-2765
2	CH_3	2972-2953
3	C-F	1000-1100
4	C=O	1725-1700

DRUG-EXCIPIENT COMPATIBILITY STUDIES

Fourier Transform Infrared (FTIR) Spectroscopy

Potential chemical interaction between drug and polymer may change the therapeutic efficacy of the drug. To investigate the possibility of chemical interaction between drug and polymer FTIR spectra of pure levofloxacin and optimised formulations were analyzed over the range 400–4000 cm^{-1}. FTIR spectra of the optimised formulations displayed all the characteristic bands of both drug and excipients, without any significant spectral shift. This suggested that there was no potential chemical interaction between the components of the formulations.

Calibration Graph of levofloxacin

The standard graph of levofloxacin has shown good linearity with R values 0.999 and 0.999 in 0.1N Hydrochloric acid and phosphate buffer pH 6.8 respectively, which suggests that it obeys the "Beer-Lambert's law" over this concentration range. The max of levofloxacin was found to be 288 nm and 288nm in phosphate buffer pH 6.8 and 0.1N Hydrochloric acid pH 1.2respectively.

In vitro drug release studies

The *in-vitro* drug release study was carried out by using dialysis bag method.

Levofloxacin niosomal suspension equivalent to 200 mg was taken in dialysis bag and the bag placed in a beaker containing 900 ml of pH 6.8 phosphate buffer. The beaker was placed over magnetic stirrer having stirring speed 100 rpm and the temperature was maintained at 37+0.5 °C. 5 ml of sample were withdrawn periodically and were replaced by fresh buffer. The samples were assayed by UV spectrophotometer at 288 nm using phosphate buffer pH 6.8 as a blank and cumulative % of drug released was calculated and plotted against time.

Kinetic data of *In vitro* Dissolution Data

The release rate kinetics data for the LNF-3. As explained by Zero order shown in figures, drug release was best followed by equation, as the plots showed higher linearity in zero order (=0.9686), Korsmeyer-Peppas (=0.6085) and Higuchi plot(r=0.9792) and first order (=0.9709).As the drug release was best fitted in the Zero order kinetics, indicating that the rate of drug release is concentration independent **.**

Accelerated stability studies

After 90 days stability studies it was found that there was no change in colour, odour and texture, in the formulations (LNF-3) .The results of stability study shown in Table 4.16 indicated that the selected formulations were stable enough at high temperature conditions (40°C) for 90 days. From the data, it shows that the formulation does not show any differences in the parameters that are evaluated and the formulation was said to be stable.

Parameters	**Time in months**			
	0 (initial)	**1st month**	**2nd month**	**3rd month**
Entrapment efficiency (%)	79.11± 3.67 Stability studies of prepared levofloxacin Niosomes (LNF-3)	79.21 ± 2.96	79.37 ± 2.78	79.11 ± 2.17
In vitro Cumulative drug release (%)	99.03 ± 0.11	99.13 ± 0.31	98.11 ± 1.01	97.2 ± 1.34

CONCLUSION

- The melting point of the drug levofloxacin was determined by using capillary method and it was found to be 224°C.
- The compatibility study was done by using FTIR spectroscopic method spectrum and resulting data revealed that there were no interactions between drug and excipients and compatible with each other.
- All the formulations of non-ionic surfactant vesicles were prepared by using reverse phase evaporation method using span 60, span80, cholesterol, chitosan and drug levofloxacin.
- Entrapment efficiency of the drug was in LNF-3 formulation was found to be 79.11 %.
- The formulation LNF-3 released 99.03% of drug for the prolonged period of time (12 hours) than other formulations.
- The formulation LNF-3 showed better drug release profile and maximum entrapment efficiency and hence it was confirmed as optimised formulation.
- The study of drug release kinetics showed that majority of the formulations governed by higuchi kinetic model. Formulation LNF-3 as highest regression coefficient value (R^2=9792) and follows drug release by higuchi kinetic model.
- The particle size of optimized noisome was sub micron size 339 nm with positive surface charge +23.1 mV.
- The prepared niosomal suspension was observed under optical microscope and

scanning electron microscope the vesicles were spherical shape.

- Accelerated stability study was conducted for the formulation by using humidity chamber by maintaining suitable temperature of 40±2ºC and relative humidity of 75±% for 3 months. The data showed that the formulation not showed larger differences in the parameters that are evaluated and the formulation said to be stable.
- From the above results it can be concluded that the LNF-3 formulation prepared with span 60 and cholesterol in the molar ratio of 100:75 showed better entrapment efficiency (79.11%) and the dug release of 99.04% for 12 hours.
- LNF-3 showed the enhanced antimicrobial activity compare to the pure levofloxacin.
- The antibacterial activities of chitosan superior in particular, against Gram-positive bacteria strains because chitosan possesses a number of poly cationic amines which can interact with the negatively charged residues of carbohydrates, lipids and proteins located on the cell surface of bacteria, which subsequently inhibit the growth of bacteria. In addition, if the molecular weight of chitosan is low, its polymer chains have greater flexibility to bind more than one cell. Thus, bridges between bacterial cells and polymer chains of chitosan are quickly formed, so that the bacteria are immediately inactivated.
- Hence levofloxacin noisome contain the chitosan, it enhance the broad spectrum antibacterial activity potency of levofloxacin.

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