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FORMULATION AND EVALUATION OF LEVOFLOXACIN LOADED SOLID LIPID NANOPARTICLES FOR SUSTAINED OCULAR DRUG DELIVERY

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ABSTRACT

The aim of the present study is an attempt to formulate and evaluate Levofloxacin loaded solid lipid nanoparticles for the sustained ocular drug delivery. Solid lipid nanoparticle was prepared by hot homogenization followed by ultrasonication method using steric acid as lipid and poloxamer 188 as surfactant. Ocular irritation studies, stability studies were conducted for the selected formulations. Compatibility studies by FT-IR showed no significant interactions between drug and excipients. The comparative *in-vitro* study of the optimized formulation shows better release than the marketed product. The developed formulation showed satisfactorily ocular tolerance and do not have produced any signs of irritations. The formulations were stable at intermediate stability testing conditions. Levofloxacin loaded SLN were successfully prepared by this hot homogenization method. The developed formulations were stable non irritant and showed better antibacterial action. From the above study we can conclude that the developed formulation is hence suitable for sustained ocular drug delivery.

Key words: Levofloxacin, Solid lipid nanoparticles (SLN), FT-IR, SEM, Ocular irritation.

INTRODUCTION

Ocular drug delivery remains challenging because of the complex nature and structure of the eye. The anatomy, physiology and biochemistry of the eye render this organ highly impervious to foreign substances. A significant challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage [1].

Acute bacterial conjunctivitis is a prevalent infection which requires an immediate work up management. Generally a treatment with ocular antibiotics is recommended to eradicate the pathogen [2]. Basically; ocular infections are treated by using topical application of antibiotics in the form of eye drops. About 90 % of the dose applied topically from such solutions is lost due to pre-corneal losses (lacrimation and drainage) which lead to poor aqueous availability, so frequent dosings are required for the instillation to achieve an adequate level and therapeutic effect [3]. To overcome these problems, various novel drug delivery systems for ophthalmic applications such as ocular inserts, collagen shields, colloidal, or particulate systems like solid lipid nano particles, nanocapsules, niosomes and liposomes have been developed to prolong the residence time and to improve the bioavailability [4].

Solid lipid nanoparticulate systems have received considerable attention over the years due to their advantages compared to other drug delivery systems. Solid lipid nanoparticles (SLN) are systems of remarkable technological relevance from a pharmaceutical perspective. These particulate systems, with sizes typically in the range of 50-1000 nm, are composed of biodegradable and biocompatible solid lipids and stabilized by emulsifier(s). These advantages include:

1. Targeted delivery of drugs to the specific site to minimize toxicity
2. Improved bioavailability by reducing fluctuations in drug levels;
3. Improved stability of drugs against enzymatic

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degradation
4. Sustained and controlled release effect that reduces dosing frequency with improved patience compliance
5. Ease of administration through various routes including topical, oral, nasal, pulmonary, intraocular, parenteral, and transdermal.

Considering the above advantages, nanotechnology has been used in ocular drug delivery to achieve extended drug release in the management of external inflammatory/autoimmune ocular diseases [5].

Levofloxacin is a broad spectrum antibiotic of the fluoroquinolones drug class, and the levo isomer of its predecessor Ofloxacin. In chemical terms, Levofloxacin, a Chiral fluorinated carboxy quinolone, is the pure (−)-(*S*)-enantiomer of the racemic Ofloxacin [6-7]. Its spectrum of activity includes most strains of bacterial pathogens responsible for various infections, including Gram negative (*E. Coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, and *P. aeruginosa*), Gram positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*) and atypical bacterial pathogens (*Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*). Compared to earlier antibiotics of the fluoroquinoline class such as Ciprofloxacin, Levofloxacin exhibits greater activity toward Gram-(+) bacteria [8-9]. Levofloxacin and other fluoroquinolones are valued for their broad spectrum of activity, excellent tissue penetration, and for their availability in topical, oral and intravenous formulations. Levofloxacin is used alone or in combination with other antibacterial drugs to treat certain bacterial infections [10-11].

Hence in the present study an attempt is made to prepare solid lipid nanoparticle based gel of Levofloxacin and to characterize the formulation for the improved ocular activity.

MATERIALS AND METHODS

Materials

Stearic acid (melting point 54°C) was purchased from fisher scientific, poloxamer 188 (melting point 52-57°C) was kindly provided by Meyer Organics (Bangalore), Levofloxacin hemihydrate was kindly provided by Embiotic laboratories (Bangalore). All other chemicals were of analytical grade or equivalent.

Compatibility studies

Drug-Excipients compatibility studies by FT-IR

To determine the drug-excipient compatibility, FT–IR studies were carried out. The IR spectra of pure drug (Levofloxacin hemihydrate), Stearic acid, Poloxamer and their physical mixture (1:1:1) were recorded by using the potassium bromide (KBr) disk technique. FT-IR measurement over the range of 4000-600 cm^{-1} was performed.

Preparation of SLN

Levofloxacin loaded SLN were prepared by homogenization method followed by ultrasonication technique. Levofloxacin, Stearic acid were dissolved in 5 ml mixture of chloroform and methanol (4:1). Organic solvents were completely removed by using a Rota-evaporator. Drug embedded lipid layer was melted by heating at 5 °C above melting point of the lipid. An aqueous phase was prepared by dissolving poloxamer 188 in deionized water and heated to the same temperature of oil phase. Hot aqueous phase was added to the oil phase, and homogenization was carried out at 10,000 rpm using homogenizer for 30 min. Coarse hot oil in water emulsion was ultrasonicated for 20 min. Levofloxacin loaded SLN were obtained by allowing hot nanoemulsions to cool to room temperature.

The composition and the formulation design of these solid lipid nanoparticulate systems is demonstrated in Table 1.

Characterization of SLN

Measurement of size and zeta potential of SLN

The size and zeta potential of SLN were measured by photon correlation spectroscopy (PCS) using Zetasizer Nano ZS (Malvern Instruments, UK). Samples were appropriately diluted with deionized water to obtain 50 and 200 Kcps for the measurements.

Scanning electron microscopy (SEM)

Surface morphology of the specimen will be determined by using a scanning electron microscope. The samples are dried thoroughly in vacuum desiccator before mounting on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 120°A Knees was coated on the sample sputter coating unit (Model E5 100 Polaron U.K) in Argon at ambient of 8-10°C with plasma voltage about 20mA. The sputtering was done for nearly 5 minutes to obtain uniform coating on the sample to enable good quality SEM images.

Drug content

Total drug content in the SLN formulation was determined by dissolving SLN formulation containing drug equivalent to 10 mg in small quantity of methanol. Then the solution was filtered through Whatmann filter paper and diluted to 100 ml with phosphate buffer pH 7.4 to give concentration 100µg/ml of Levofloxacin. Then 1 ml was pipetted out in 10 ml volumetric flask to give a concentration 10 µg/ml and then absorbance was measured using UV Spectrophotometer at λmax 288 nm against blank.

Entrapment efficiency

The entrapment efficiency (EE %) of Levofloxacin loaded SLN was determined by centrifugation method. 2 ml of nanosuspension was taken and subjected to

centrifugation on a cooling ultracentrifuge at 5000 rpm for 30 min. The clear supernatant was siphoned off to separate the unentrapped drug. 1 ml of supernatant was taken and diluted with methanol up to 10 ml and absorbance was recorded at 288 nm using UV spectrophotometer. Amount of drug present in supernatant and sediment gave a total amount of drug present in system. The % entrapment was determined by following formula:

$$Percentage\ entrapment = \frac{Amount\ of\ drug\ in\ sediment}{Total\ amount\ of\ drug\ added}\ X\ 100$$

In-vitro drug release studies

The release of drug was determined by using the treated cellophane membrane mounted on the one end of open tube, containing drug equivalent to10 mg of formulation. The dialysis tube was suspended in 250 ml beaker, containing 200 ml PBS (pH 7.4). The solution was stirred at 200 rpm with the help of magnetic stirrer at 37±0.5 °C. Perfect sink conditions were maintained during the drug release testing. The samples were withdrawn at suitable time interval (at 1, 2, 4, 6, 8, 12, 18 and 24 hrs). The dissolution medium was replaced with same amount of fresh PBS (pH 7.4) solution to maintain the volume 200 ml throughout the experiment. The drug content in the withdrawn samples (1 ml) were estimated at 288 nm after making the volume up to 10 ml with PBS (pH 7.4) and cumulative % of drug released was calculated and plotted against time (t).

Preparation of Levofloxacin loaded SLN gel

Gels were prepared by cold mechanical method, by using Carbopol 934 as gelling agent, triethanolamine as neutralizing agent and water as dispersion medium.

Method of preparation

Required quantity of Carbopol 934 was taken and hydrated in sufficient quantity of water for 24 hrs. Further, the hydrated gel was stirred for 4 hrs. Triethanolamine was added for the neutralization of gel and stirred it until a clear transparent gel was obtained. Accurately weigh the SLN which is equivalent to 100 mg of Levofloxacin and incorporated into 5 gm of gel by mechanical mixing for 2 hrs until it gets distributed uniformly.

Evaluation parameters for the SLN gel

Physical examination

Macroscopic examination for visual appearance, color, and clarity was done for the prepared gel.

Measurement of pH

The pH of gel was determined by using digital pH meter. The electrode first calibrated with pH 4.0 and pH 7.0 solutions then the measurement of pH of gel was done.

Spreadability

The Spreadability of the gel was determined using the following technique: 0.5 gm gel was placed within a circle of 1 cm diameter premarked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5min. The increase in the diameter due to spreading of the gels was noted.

Viscosity and Rheological properties

Viscosity of the SLN gel was done by using Brook field viscometer with T-bar spindle (no. 94). Gel was filled in a beaker of suitable size and spindle was lowered perpendicularly and rotated at such a speed so as to generate torque >30%. The viscosity of gel was obtained by multiplying the viscometer reading with multiplication factor given in Brookfield viscometer catalogue.

Drug content

The drug content was determined by 2 gm SLN gel sample was withdrawn from container and dissolved in methanol and made volume up to 100 ml. After suitable dilution absorbance was measured by UV spectrophotometer against blank at λmax 288 nm and the drug content was calculated.

***In-vitro* release studies**

The release of drug was determined by using the treated cellophane membrane mounted on the one end of open tube, containing 1gm of SLN gel. The dialysis tube was suspended in 250 ml beaker, containing 200 ml PBS (pH 7.4). The solution was stirred at 200 rpm with the help of magnetic stirrer at 37±0.5°C. The diffusion cells were maintained at 37±0.5 °C with stirring at 200 rpm throughout the experiment. The samples were withdrawn at suitable time interval (at 0.5, 1, 2, 4, 6, 8, 12, 18 and 24 hrs). The dissolution medium was replaced with same amount of fresh PBS (pH 7.4) solution to maintain the volume 200 ml throughout the experiment. The drug content in the withdrawn samples (1 ml) were estimated at 288 nm after making the volume up to 10 ml with PBS (pH 7.4) and cumulative % of drug released was calculated and plotted against time (t). Simultaneously the release studies compared with the marketed product (Levobact-0.5% ophthalmic solution).

Ocular irritation studies

Ocular irritation studies were performed on male albino rabbits weighing 1-2kg according to the Draize technique. Little amount of the sample is placed in the lower cul-de-sac of the eye and irritancy was tested at the time interval of 1 hr, 2 hrs, 48 hrs, 72 hrs, and one week after administration. The rabbits were observed periodically for redness, swelling and watering of the eye [12].

Stability studies

Whenever a new formulation is developed, it is very essential to establish that the therapeutic activity of the drug has not undergone any change. To conform this,

the selected formulations were subjected to stability studies. Intermediate stability testing studies was performed for 6 months. The optimized formulations were kept at 30±2 °C and 65±5% RH. Drug entrapment and drug release were fixed as physical parameters for stability testing [13].

RESULTS AND DISCUSSION

Compatibility studies

FTIR studies to find out the compatibility of drug with the excipients

The FT-IR spectra of pure Levofloxacin and the physical mixture (1:1:1) of drug with stearic acid and poloxammer188 given in Fig.1 and Fig.2 respectively. The IR spectra of pure drug shows principal peaks at 1724 cm^{-1} (C=O stretching vibration of – COOH group), 1294 cm^{-1} (C-N stretching), 1084 cm^{-1} C-F (Stretching). The physical mixture on the other hand shows peaks at 1710, 1290 and 1097 cm^{-1}. From this we have concluded that the physical mixture of drug, Levofloxacin does not show any major interactions with the formulation ingredients Stearic acid, poloxamer 188.

FT-IR studies for the optimized formulation F4 was carried out and from the spectra, we have observed that the absence of characteristic peaks of the pure drug which indicates the drug was encapsulated in the lipid core of solid lipid nanoparticles shown in Fig.3.

Drug content and entrapment efficiency

The percentage of drug content and entrapment efficiency of Levofloxacin in different SLN formulations with was found spectrophotometrically. The results are shown in the Table 2.

Highest entrapment efficiency was observed in F3 and F4 with 90.23% and 95.64% respectively. The high drug entrapment may be observed due to the rapid quenching of the drug occurred in the lipid phase due to the presence of Poloxamer as surfactant phase and it was observed that increase in the lipid content resulting increase in the entrapment efficiency.

Particle size analysis

Particle size of the solid lipid nanoparticle was analyzed by using Malvern particle size analyzer (Zetasizer V.7.03) for the optimized formulations. The results showed that the mean particle size measured by dynamic light scattering technique was 366 nm to 392 nm (Table.2) for Levofloxacin loaded SLN. This indicates that the formulations fell in well acceptable nano range. Formulation F1 showed mean particle size of 366 nm where as formulation F4 showed mean particle size of 392 nm, this may be attributed to increase in the lipid content in the formulation F4.

Zeta potential measurement

Zeta potential is a key factor for evaluation of the stability of colloidal dispersion. The zeta potential was measured for the optimized formulations. The values of zetapotential of Levofloxacin loaded SLN was ranged from -20.6 to -26.6 mV (Table.2) were sufficient to keep the particles stable.

Scanning electron Microscopy

The shape and surface morphology of optimized SLN formulation (F4) was studied by SEM. The microphotographs reveal that the particles are uniform in size and roughly spherical in shape (Fig.4). The presence of aggregates might be attributed to a short redispersion time after centrifugation and drying at room temperature.

In-vitro release studies

In-vitro release study of Levofloxacin from various formulations was conducted for 24 hrs by using dialysis membrane. Cumulative % drug release was plotted against time. All the formulation showed more than 30 % in the first 1 hr due to the presence of un-entrapped drug and the drug entrapped on the surface of lipid core which released faster showing dose dumping which is suitable to produce the initial effect of drug. It has been found that from the SLN formulation, the release were F1- 95.28%, F2- 88.14 %, F3-85.26% and F4 - 78.81%. The increase in lipid ratio from F1 to F4 causes decrease in the drug release and the release was more controlled by increasing the lipid ratio. The results are shown in Fig.5.

Preparation and Evaluation of Levofloxacin loaded SLN gel

SLN gel was prepared by using carbopol 934 as gelling agent using cold mechanical method (table). Optimized formulation of SLNs was selected based on their zeta potential, particle size, %EE, *in-vitro* drug release and incorporated into gel. Formulation F4 showed better results among all formulations, so formulation F4 selected as best formulation further it is converted into gel and coded as GF4.

Evaluation of SLN gel

Physical examination

The prepared SLN gel (GF4) was off-white in color and homogenous.

pH

The pH of the SLN gel (GF4) was found to be 7.2.

Spreadability

The Spreadability of the gel (GF4) was found to be 10.12 g/sec.

Viscosity

Viscosity of the SLN gel was done by using Brook field viscometer with T-bar spindle (no. 94). Viscosity of the SLN gel (GF4) was found to be 10945±80 cps.

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***In-vitro* drug release studies**

The direct exposure of SLN dispersion to diffusion media and quick release of drug may account for rapid initial release in SLN dispersions. SLN-gel formulation showed controlled drug release over 24 hrs.

Kinetic model data analysis

The release data obtained from formulation GF4 was subjected for data analysis. The formulations followed Peppas order release profile and the 'n' value was found to be less than 0.5. This indicates that the release approximates Fickian diffusion mechanism.

Comparative *In vitro* release studies

Comparative *in vitro* release studies were conducted for formulation GF4 with the marketed product Levobact 0.5% ophthalmic solution and the results showed that formulation GF4 showed extended release over a period of 24hrs. The results are shown in Fig.6.

Ocular irritation study was conducted for formulation GF4 as per Draize technique in male Albino rabbits. The results of the ocular studies indicated that the formulation GF4 was non-irritant with excellent ocular tolerance. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible. Only a few signs of increased lacrimation were noted. The results are shown in tables 4 to 6.

Stability studies

Stability studies of Formulation F4 and GF4 showed that there was there is very slight change occurred in the drug release and drug content, which is negligible when compared to initial values. From this we have observed that the formulations were stable on storage. The results are shown in tables 7 and 8.

Table 1. Formulation Design for the Preparation of Levofloxacin loaded SLN

Ingredients in % w/v	F1	F2	F3	F4
Levofloxacin	0.5	0.5	0.5	0.5
Stearic acid	0.5	1.0	1.5	2.0
Poloxamer 188	0.5	0.5	0.5	0.5
Chloroform (ml)	4	4	4	4
Methanol (ml)	1	1	1	1
Purified Water (ml)	100	100	100	100

Table 2. Evaluation parameters for the Levofloxacin loaded SLN

Formulation Code	%EE	Size (nm)	Poly Dispersity Index	Zeta Potential (mv)
F1	55.86±0.40	366	0.392	− 20.6
F2	68.34±0.28	368	0.346	− 22.4
F3	90.23±0.50	386	0.286	−26.2
F4	95.64±0.37	392	0.342	−26.6

Table 3. Regression co-efficient (r^2) value of different kinetic models for optimized formulation gel (GF3)

Optimized formulation Gel	Zero order	First order	Korsmeyer-Peppas	'n' value	Higuchi
Regression co-efficient(r^2)	0.984	0.956	0.989	0.342	0.980

Table 4. Eye irritation testing: Rabbit conjunctival observation

Redness	Normal rating	Rating for formulation (GF4)
Vessel normal	0 none	0
Vessels definitely injected above normal	1 slight	0
More diffuse, deeper crimson red with individual vessels not easily discernible	2 moderate	0
Diffuse beefy red	3 severe	0

Table 5. Eye irritation testing: Rabbit iris observations

Values	Normal rating	Rating for formulation (GF4)
Normal	0 none	0
Folds above normal, congestion, swelling, iris reacts to light	1 slight	0
No reaction to light, hemorrhage, gross destruction	2 severe	0

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Table 6. Eye irritation testing: Rabbit corneal observations for opacity and area of cornea involved

Opacity	Normal rating for opacity	Rating for formulation GF4	Area of cornea involved	Normal rating for corneal area involved	Rating for formulation GF4
No opacity	0 none	0	25% or less (not 0)	1	0
Diffuse area details of iris clearly visible	1 slight	0	25% to 50%	2	0
Easily visible translucent areas, details of iris slightly obscure	2 mild	0	50% to 75%	3	0
Opalescent areas, no details of iris	3 moderate	0	Greater than75%	4	0
Opaque, iris is invisible	4 severe	0	-	-	0

Table 7. Stability studies for the formulation F4

Temperature And RH	% Drug Entrapment After (months)				% Drug Release After (months)			
30±2 °C and 65±5% RH	0	1	3	6	0	1	3	6
	95.74±0.24	94.89±0.38	92.23±069	90.48± 019	85.26	85.26	83.26	80.48

Table 8. Stability studies for the SLN gel GF4

Temperature and RH	Parameters	After (months)			
		0	1	3	6
30±2 °C and 65±5% RH	pH	7.2	7.2	7.2	7.16
	Drug content	95.20±0.14	95.09±0.59	93.23±0.64	90.29±00.39
	Drug release	84.12	84.09	82.16	79.96

Figure 1. FTIR spectrum of Levofloxacin

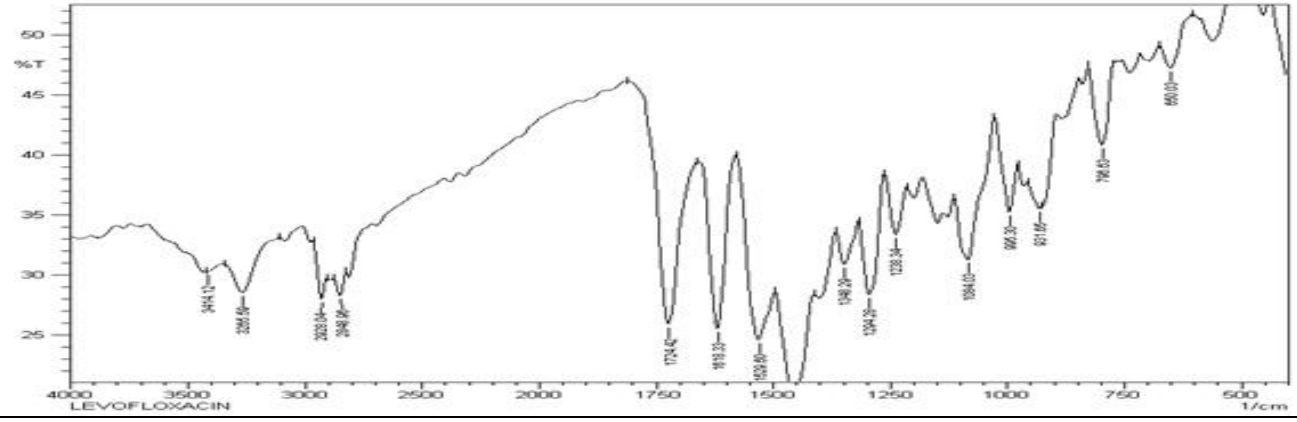

Figure 2. FTIR spectrum of Levofloxacin with excipients

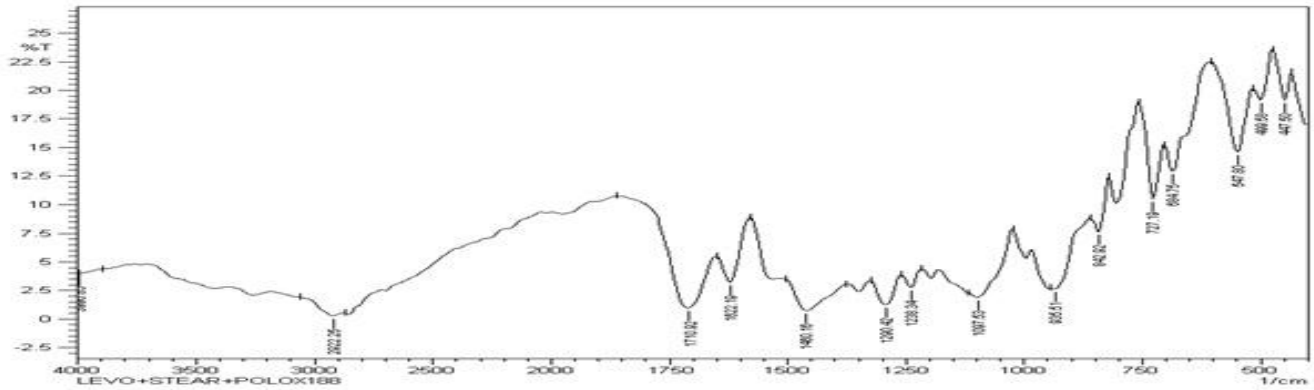

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Figure 3. FTIR spectra of optimized formulation F4

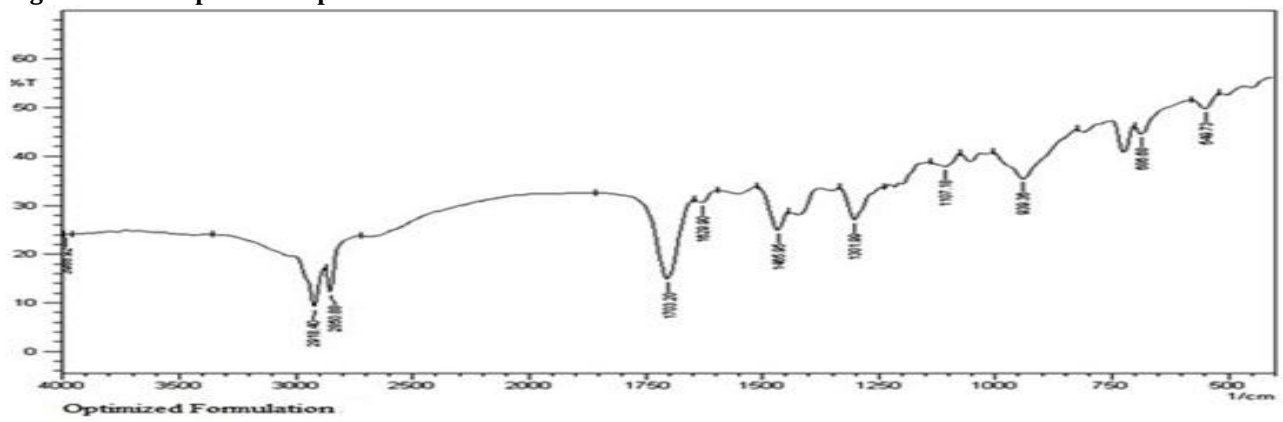


Figure 4. SEM of the optimized formulation F4

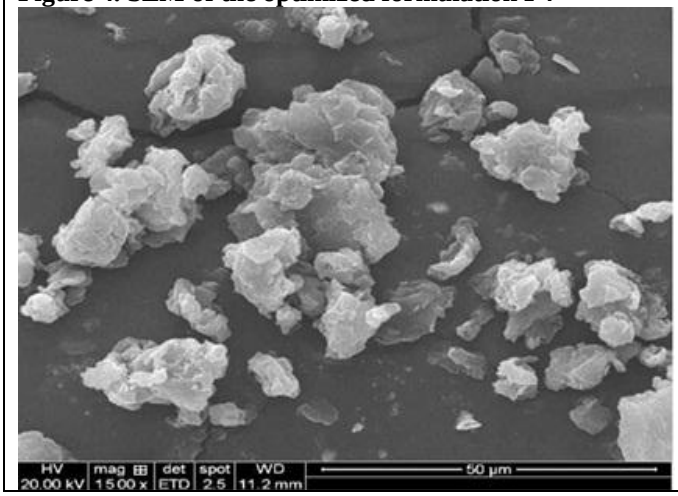
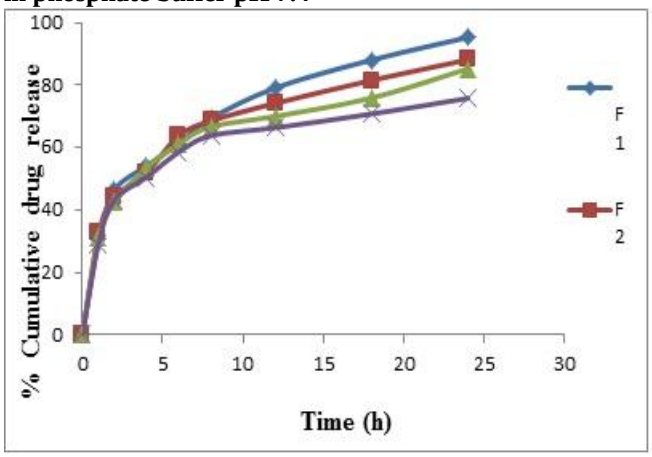


Figure 5. *In-vitro* release profile of formulations F1 – F4 in phosphate buffer pH 7.4



CONCLUSION

Hot homogenization followed by ultra sonication method is suitable to produce SLN in nanometric size range. The drug Levofloxacin could very well be entrapped in the solid SLN and their characteristics could be monitored by making changes in various formulation and process variables. The Levofloxacin loaded SLN gel showed an extended release when compared to marketed formulation. Ocular irritation studies revealed that the developed formulation do not have produced any ocular irritation. From the stability studies we observed that the formulations were stable at intermediate stability testing conditions. The developed formulation is hence suitable for sustained ocular drug delivery.

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