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Research Article

# Formulation Development and Characterization of Nanostructured Hetrolipid Matrix of Levofloxacin Hemihydrate for Ocular Drug Delivery

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- Entrapment efficiency

**Abstract**

Solid lipid nanoparticles (SLNs) formulated using one type of lipid (homolipid) suffer from low drug encapsulation and drug bursting due to crystallization of the lipid into the more ordered β modification, thus leading to decreased drug entrapment and faster drug release. This study assessed the feasibility of using nanostructured lipid matrices (NLM) for ocular delivery of levofloxacin hemihydrate adopting heterolipids composed of mixtures of lipid and stabilized by surfactant. The systems were prepared using the modified solvent injection method followed by ultrasonication. The NLM were characterized by entrapment efficiency percentages EE %), mean particle size (PS), polydispersity index (PDI), and zeta potential (ZP). The *in vitro* drug release was studied using dialysis bag method. Decreasing gelucire concentration was accompanied by an increase in drug entrapment followed by an increase in particle size. The best formula, composed of Compritol: Gelucire in 1:1 ratio, 10 mg stearylamine, and 1% poloxamer, with EE% of 69.41%, PS of 220.1 nm, PDI of 0.202, and ZP of 24.3 mV, showed *in vitro* sustained release properties for 24 hours.

## ABBREVIATIONS

**SLN:** Solid Lipid Nanoparticles; **NLC:** Nanostructured Lipid Carrier; **NLM:** Nanostructured Lipid Matrix; **CSA:** Cetostearyl Alcohol; **SA:** Stearylamine

## INTRODUCTION

Conventional ocular drug delivery systems such as eye drops suffer from very poor bioavailability because the eye is protected by complicated defense mechanisms that make it difficult to achieve an effective drug concentration in the target area. Colloidal drug delivery systems such as liposomes and emulsions have been developed to solve this problem but they suffer from poor stability [1]. The field of nanotechnology is one of the most active research areas in modern materials science. Nanoparticles exhibit new or improved properties based on specific characteristics such as size distribution and morphology [2]. Nanoparticles, primarily developed for i.v administration, were first proposed for ophthalmic drug delivery in 1981. Gurny and co-workers (1981) first indicated the potential advantages of nanoparticles (named pseudo-latexes) over aqueous polymer solutions [3,4].

Polymeric nanoparticles have been intensely investigated since their introduction by Speiser and co-workers in the mid-seventies [3]. Despite their interesting properties, not many products made it to market because of the presence of solvent residues left over from production, the cytotoxicity of the polymers and the lack of low-cost, qualified large scale production units yielding a product of a quality acceptable by the regulatory authorities.

In the 90's, a new type of carrier was developed, the so-called solid lipid nanoparticles (SLN) which have been studied and modified from the original concept into more complex systems (eg, nanostructured lipid carriers or lipid-drug conjugate) but basically the usage of lipid materials to form the carrier has presented many advantages over other kinds of materials. SLN are submicron-sized carriers composed of a lipid solid matrix stabilized by a surfactant. Such systems have some advantages

over other colloidal carriers (nanoemulsions, microparticles, polymeric nanoparticles, liposomes etc.), such as low toxicity, high drug payload, capability of including lipophilic and hydrophilic drugs, drug targeting, controlled release (fast or sustained) and occlusive properties [5].

SLN formulated from a single type of lipid (homolipid) suffer from low drug encapsulation and possible drug bursting due to crystallization of the lipid into the more ordered β modification, which shows a high degree of order and limited imperfections in the crystal lattice. The use of mixtures of lipids, which do not form a highly ordered crystalline arrangement, is needed to overcome these limitations. Such a lipid matrix could be achieved by using a combination of solid lipid and oil, which is termed a nanostructured lipid carrier (NLC), or through the use of a solid lipid mixture (heterolipids) with a complex nature that includes different chain lengths and melting points, which is called a nanostructured lipid matrix (NLM). Mixing lipids modifies the polymorphic properties of the single lipid and has been proven to produce lipid matrices of low crystallinity [1].

SLN can be produced by many methods, although the following are the most common: i) hot high pressure homogenization (HPH) technique and cold HPH technique; ii) solvent emulsification/evaporation (SEE); iii) high shear homogenization (HSH) and/or ultrasound and iv) microemulsion [5].

Bacterial conjunctivitis is a relatively common infection and affects all people, although a higher incidence is seen in infants, school children and the elderly [6]. Bacterial conjunctivitis is commonly treated empirically with broad spectrum antibiotics. Broad spectrum antibiotics that have good efficacy against both gram negative and gram positive are necessary as a diverse range of pathogens can be the cause of infections. The antibiotics are given in form of eye drops or eye ointment. The ointments are less prescribed because of blurring vision. In moderate to severe infections, or antibiotic resistant infections and in immune compromised patients, fluoroquinolones are recommended.

Levofloxacin, a third generation fluoroquinolone antibacterial agent, has a broad spectrum of activity against gram positive and gram negative bacteria. Chemically, Levofloxacin, a chiral fluorinated carboxyquinolone, is the pure (-)-(S) - enantiomer of the racemic drug substance ofloxacin. It is active against both penicillin susceptible and penicillin resistant Streptococcus pneumoniae. It inhibits bacterial DNA gyrase and topoisomerase IV. Levofloxacin is safe and generally well tolerated in comparison with some other quinolones [7].

Various formulations containing levofloxacin for ophthalmic delivery have been prepared using different preparation methods, optimized and studied by some research groups around the world. pH- triggered In Situ Gelling System [8], poly(lactic acid)/poly(lactic-co-glycolic acid) (PLGA) Nanoparticles [9], Chitosan Nanoparticles [10], Ion Activated In situ Gelling System [6], Niosomal In Situ Gel [11] have been prepared and optimized.

The present study was aimed at formulating solid lipid nanoparticles of levofloxacin hemihydrate. The objective of this study was to assess the feasibility of using NLM for ocular delivery of Levofloxacin using heterolipids composed of mixtures of different lipids stabilized by surfactants. The NLM were prepared using the solvent injection method followed by ultrasonication. The use of heterolipids was expected to create imperfections in the crystal lattice, providing more space that can localize more drug and thus improve the entrapment efficiency. Formulations using combination of Compritol with Gelucire, Compritol with Cetostearyl alcohol (CSA) and CSA with Gelucire were prepared. The CSA-Gelucire and Compritol-Gelucire combination which showed better entrapment and *in-vitro* release were tested for particle size and polydispersity index.

## MATERIALS AND METHODS

### Materials

Levofloxacin hemihydrate was obtained as a gift sample from FDC Limited, Mumbai. Compritol ATO 888 and Gelucire 44/14 were kindly gifted by Gattefosse. Stearylamine was received as gift sample from Indoamines, Mumbai. Poloxamer 188 was obtained as gift sample from BASF. Cetosteryl Alcohol (CSA) L.R grade, Isopropyl Alcohol L.R grade, Potassium dihydrogen orthophosphate A.R grade and Sodium hydroxide A.R grade were purchased from S D Fine Chemical Limited, Mumbai.

### Method of preparation

Cationic Levofloxacin-loaded NLM were prepared by solvent injection method followed by ultrasonication. The total amount of the lipid phase in the developed formulations was kept constant at 400 mg. The method involved preparation of organic phase and aqueous phase. The organic phase comprised of lipid and drugs dissolved in iso propyl alcohol. The aqueous phase was prepared by dissolving surfactant i.e. poloxamer 188 in distilled water. The aqueous phase was heated on water bath to 85˚C the organic phase was heated 5˚C above the melting point of lipid. The organic phase was injected in aqueous phase under stirring using hypodermic needle and stirred at 3000 rpm for 20 min using mechanical stirrer. The mixture was sonicated further for 15 min using probe sonicator and cooled rapidly in ice bath to obtain solid lipid nanoparticles. The nanoparticles were stored in refrigerator till further use. All the operations were performed in aseptic area under laminar flow using presterilized drug and excipients.

## CHARACTERIZATION OF NANOPARTICLES

### Entrapment efficiency and drug loading

The entrapment efficiencies of prepared system were determined by measuring the concentration of free drug in the dispersion medium [12]. The entrapment efficiency was performed using dialysis bag (molecular weight cut-off 12000-14000). 3 mL of NLM dispersion first poured into dialysis bag with two ends fixed by thread and placed into 50 mL distilled water. The absorbance of the system was noted after fixed time. The amount of free drug was calculated.

$$\text{Entrapment Efficency} = \frac{W_t - W_f}{W_t} \times 100$$

$$\text{Drug loading} = \frac{W_t - W_f}{W_t - W_f + W_l} \times 100$$

Where $W_t$ is weight of total drug

$W_f$ is weight of free drug

$W_l$ is weight of lipid

### Particle Size and Polydispersity Index

The particle size and polydispersity index of optimum NLM dispersions were measured using Zetasizer (Malvern Instruments Ltd., UK). The sample was placed in disposable sizing cuvette without dilution to obtain the results.

### Zeta potential

The Zeta potential of optimum NLM dispersions was measured using Zetasizer (Malvern Instruments Ltd., UK). The sample was placed in clear disposable zeta cell without dilution to obtain the results.

### *In-vitro* release

*In vitro* release studies were performed using the dialysis bag method [12]. It was modified to maintain a sink condition and achieve satisfactory reproducibility. The dissolution medium was freshly prepared phosphate buffer pH 7.4. The dialysis bag was previously soaked overnight in distilled water and was tied to both ends after filling NLM dispersion into it. The dialysis bag was suspended in dissolution medium maintained at 37 ± 2°C. The dissolution medium was shaken at 50 rpm using mechanical shaker. Aliquot, each of 5 mL was withdrawn at fixed interval of time and the same volume of fresh medium was added accordingly. The absorbance of sample was taken at 288 nm.

## RESULTS AND DISCUSSION

The UV spectrophotometric analysis method used for the *in vitro* assay was developed and validated and was found to be linear over the range of 1–10 µg/mL, while the limit of detection and limit of quantification were found to be 0.02657 and 0.08051 respectively. The regression equation was y = 0.1309x - 0.0078 where *y* is the absorbance and x is the concentration of levofloxacin in µg/mL (*r* = 0.9993).

Levofloxacin loaded NLM were successfully prepared through the use of solvent injection method followed by ultrasonication. Heterolipid mixtures of CSA–Gelucire, Gelucire–Compritol and CSA–Compritol were used.

Various trials were carried out to determine the best combination of lipids. CSA–Gelucire, Gelucire–Compritol showed better entrapment and *in vitro* release of drug than CSA – Compritol. These combinations showed 60-70% of the drug entrapment and release of the drug up to 24 h.

Tween 80 and Poloxamer 188 were the surfactants of choice because of their non-ionic character and excellent ocular tolerability. But entrapment and *in vitro* release of drug from NLM prepared using Poloxamer 188 was better than Tween 80. Formulation prepared using Poloxamer 188 as a surfactant showed 60-70% of the drug entrapment and release of the drug up to 24 h.

Stearylamine (SA) has been commonly used in preparing cationic emulsions intended for ocular drug delivery and was found to be non-irritating with no evidence of inflammatory or toxic response when instilled in rabbits' eyes. SA was chosen to serve multiple roles. The first of these was to impart a positive charge to produce cationic SLN that could elicit electrostatic adhesion or interaction with the negatively charged mucin of the corneal epithelium, hence increasing ocular contact time and providing prolonged release. SA also acts as a co-surfactant for further particle size reduction.

### Entrapment efficiency and drug loading

The entrapment efficiency and drug loading of formulations varied from 66.27 % to 72.49 % and 1.62 % to 1.78 % respectively. The highest entrapment and drug loading was seen in formulation F3 and lowest in F5. As the amount of gelucire decreases, there is an increase in the entrapment efficiency and drug loading. Thus it could be assumed that by using combination of lipids there would be defects in crystal lattice, which would have resulted in an increase in the entrapment of drug. Entrapment of the drug varies as the ratios of lipids are varied. It might be due to the increased viscosity of the medium, because increasing the amount of compritol resulted in faster solidification of the nanoparticles. This would also prevent drug diffusion into the external phase of the medium [12]. Table 2 shows the entrapment efficiency and drug loading of various formulations.

### Particle size and polydispersity index

The results of particle size and polydispersity are shown in table 3. All the NLM formulation showed mean particle size below 300 nm. The average particle size ranged from 116.3 nm to 280.4 nm. PdI value ranged from 0.202 to 0.367. As the amount of gelucire in the formulation increases there was a relative decrease in average particle size. The increase in particle size would be due to the deposition of other lipid on the surface of solidified compritol, as compritol has higher melting point, it would start solidifying first when it is cooled in an ice bath compared to other lipid. As the amount of gelucire in formulation decreases there was an increase in entrapment which might also be the reason of larger particle size.

### *In-vitro* release

The % cumulative releases of formulations are shown in figure

**Table 1:** Composition of formulations.

| Ingredients | Formulations | | | | |
|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 |
| **Gelucire 44/14 (mg)** | 300 | 200 | 100 | 200 | 300 |
| **Cetosteryl alcohol (mg)** | 100 | 200 | - | - | - |
| **Compritol ATO 888 (mg)** | - | - | 300 | 200 | 100 |
| **Stearylamine(mg)** | 10 | 10 | 10 | 10 | 10 |

**Table 2:** Entrapment efficiency and drug loading of Formulations.

| Formulation | Entrapment Efficiency (%) | Drug loading (%) |
|---|---|---|
| **F1** | 68.8057 | 1.69105 |
| **F2** | 72.0143 | 1.76852 |
| **F3** | 72.4981 | 1.78019 |
| **F4** | 69.4169 | 1.70582 |
| **F5** | 66.272 | 1.6298 |

1 and 2. The common feature in these profiles was an initial burst effect with approximately 33.71%, 28.27%, 33.52%, 54.22% and 49.30% of drug being released in the first hour for F1, F2, F3, F4 and F5. This was followed by a slower exponential release of the remaining drug which released over the following 24 hours. The initial burst effect could be attributed to the portion of the drug that was positioned on the outer shell of the NLM. It was followed by the slower release of the remaining drug that was entrapped inside the core of the NLM. The presence of the drug in the outer shell of the NLM could be due to reduction in drug solubility in the aqueous phase during solidification of NLM. This forces the drug to go to the lipid phase that has already been recrystallized and so is prevented from entering the lipid core, which means that it is concentrated on the surface of the NLM. The release data were fitted to various kinetic models in order to calculate

**Table 3:** Particle size and Polydispersity index of formulations.

| Formulation | Av. particle size (nm) | PdI |
|---|---|---|
| **F1** | 116.3 | 0.251 |
| **F2** | 217.8 | 0.219 |
| **F3** | 280.4 | 0.224 |
| **F4** | 220.1 | 0.202 |
| **F5** | 140.5 | 0.367 |

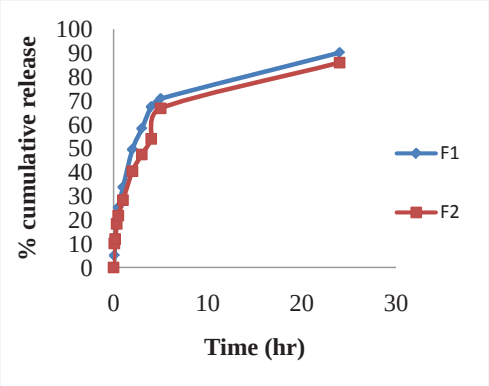


**Figure 1** % cumulative release of Levofloxacin from formulations F1, F2 prepared using CSA and Gelucire.

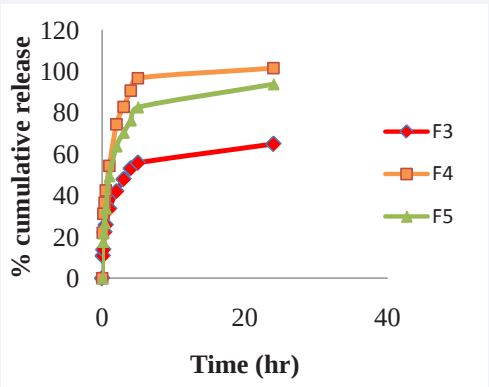


**Figure 2** % cumulative release of Levofloxacin from formulations F3, F4, and F5 prepared using Compritol and Gelucire.

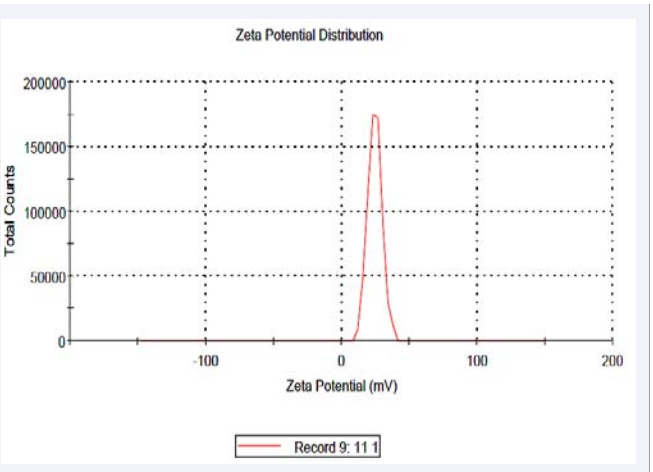


**Figure 3** Zeta potential distribution of formulation F4.

the release constant and coefficient of determination ($R^2$). Among the model tested, the drug release profiles of formulations F1, F2, F3, F4 and F5 were best fitted in Higuchi Matrix model based on the regression coefficients 0.9913, 0.9916, 0.9778, 0.9914 and 0.9854 respectively. The linearity of the plot indicated that the release process was diffusion controlled. Thus, the amount of drugs released was dependent on the matrix drug load. The release exponent (n) of formulations indicative of non- fickian drug release.

### Zeta potential

The formulation for measurement of zeta potential was selected on the basis of results obtained from particle size analysis, entrapment efficiency and *in vitro* release. Formulation F4 was selected which had lowest particle size with high entrapment efficiency and showed release of drug up to 24 h. The zeta potential obtained was 24.3 mV. Zeta potential above 25mV is generally considered to be stable formulation. The result obtained is very much near to it which shows formulation would remain stable

## CONCLUSION

SLN were successfully prepared using combinations of different lipids and were characterized by mean particle size below 300 nm for all the formulations using Poloxamer 188 as surfactant. Formulation F4 showed low particle size, low PdI, high drug entrapment and better release. Decreasing gelucire concentration was accompanied by an increase in Levofloxacin hemihydrate entrapment into the nanoparticles. Increasing gelucire concentration resulted in decrease in particle size. Thus an optimum concentration of gelucire with other lipid must be used to obtain higher entrapment of drug and low particle size of nanoparticles. The *in vitro* release data showed that the gelucire, compritol combination showed release of the drug upto 24 h than gelucire, CSA combination. The release pattern also varied with the change in the concentration of the lipids. It can be concluded that the controlled release once a day formulation of levofloxacin hemihydrate can be prepared for treating bacterial conjunctivitis with better patient compliance and therapeutic effect.

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