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Preformulation Studies of Livofloxacin as Nanoemulsion for Ocular Drug Delivery

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Abstract

Objective: Levofloxacin is the L-isomer of the racemate ofloxacin, a quinolone antimicrobial agent. The objective of this article is to exhibit the quantitative methods used to determine consistency in developmental research. Author represented methods of preformulation for Levofloxacin as ocular nanoemulsion capable of delivering the drug in a sustained manner, thus avoiding frequent instillation of the drops which may induce toxic side effects and cellular damage at the ocular surface. Preformulation studies are essential to make sure the development of established as well as therapeutically safe and effective dosage form. **Material and Methods:** The preformulation studies, performed in this research include identification of drug, solubility analysis, and partition coefficient and drug compatibility. In current work entire preformulation study was carried out, which contain identification of drug, quantitative estimation of drug, solubility determination, melting point determination, partition coefficient determination, Screening etc. **Results:** The melting point of Levofloxacin was found to be 223–228°C. The log *P* value was found to be-0.34 ± 0.05, from which it can be interpreted that drug is highly Lipophilic in nature. The scanned λ_{max} was found to be 298 nm. No significant changes were found when FTIR spectra of physical mixture compared with FTIR spectra of pure drug and excipients. This indicates the absence of any possible interaction between the drug and excipients which confirms the identity and purity of drug. **Conclusion:** These results propose that Levofloxacin serves as appropriate candidate for the ocular drug delivery system.

Keywords: Levofloxacin, Nanoemulsion, Physicochemical characterization, Preformulation, Screening, Solubility

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Introduction

Levofloxacin belongs to the fluoroquinolone (flor-o-kwin-o lines). It is a broad-spectrum antibacterial of the third generation Fluoroquinolones class of drug along with half life is 6–8 h.[1] It is the L-isomer of the racemate ofloxacin and quinolone antimicrobial agent. It is chemically, chiral fluorinated carboxyquinolone. It is natural(S)- enantiomer of the racemic mixture substance ofloxacin.[2] Levofloxacin is used for the best tissue permeation and formulation such as as oral, intravenous, and topical drug delivery also used frequently in eye infections. Levofloxacin availability for various combinations along with another antibacterial drugs.[3-7] it is a group of antibiotic and it used to treatment of skin disease, for example., prostate, bladder, kidney, and sinus. It cures the body bacterial infection. Therapeutically used to cure tuberculosis, diarrhea, endocarditis, anthrax, urinary tract prostate, respiratory tract, and plague or can be administered by the ocular system. Levofloxacin is effective across the Gram-positive bacteria and Gram-negative bacteria. Such that topoisomerase IV and DNA gyrase impending by function of quinolones. To isolate the replicated DNA, Topoisomerase IV is required point to division of cell in bacteria and if no more isolated, then it stops the whole process. DNA gyrase helps in great coiling of DNA, it makes to able new cell prepared. Levofloxacin acts as a bactericide.[8]

Ocular drug administration this medication technique helps the administered ophthalmic drug by targeted area of site of ocular passage in its maximum retained concentration providing relief. The complex function, anatomy and physiology of the eye, such as, i.e., Cornea's impermeability, inner and outer blood-retinal barrier, nasolacrimal drainage system, limitations of non-corneal structures, pose a challenge to ocular drug administration as; it makes the bioavailability of ophthalmic drug rare by diluting the concentration of administered drug. Basis on above feature of the eye, significant advances and research are been carried to optimize the ophthalmic drug remittance negating the ocular barriers.[9-13]

The term nanoemulsion is made from two words, i.e., nano or emulsion, also called nanometer.[14] Nanoemulsion is made up of two non-miscible liquids, and that are heterogeneous system. Nanoemulsion is stabilized with co-surfactant and surfactant. It contains two non-miscible liquids substances. It is thermodynamically and kinetically stable formulation.[15-17] Nanoemulsion contains essential oils the essential oils exceed the capability of hydrophobic drugs which are not soluble in the essential oils instance.[18] Nanoemulsions have stability against creaming, flocculation, coalescence, sedimentation. It is safe approved by the food drug administration. It is non-irritant and non-toxic use, generally recognized as safe.[19,20] nanoemulsion is active medicament administration implement this is only because of higher solubilizing drug substance, stability for long duration of period, and effective emulsification.[21]

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Materials and Methods

Materials

Various materials have been procured from standard suppliers. All chemical reagents used were analytical grade. Drug sample was obtained as a Gift sample from MMG Healthcare Ltd. Sirmour (H.P.) India. Acetic Acid (Finar Reagent-company), Castor Oil, Tween 80, Methanol, (HPLC Grade), Potassium bromide (KBr), Mathanol (AR), Sodium chloride, Calcium chloride, Sodium bicarbonate, Acetonitrile, Triethylamine (SDFCL-Company), Olive Oil, Dialysis Membrane, Sodium Dihydrogen Phosphate (HiMedia-company), Iso Propyl Myristate, n-Octanol, Soya lecithin (CDH-company).

Preformulation Studies

Preformulation studies were performed for physicochemical properties of active compounds, provide an insight of the development of an efficacious, stable, and safe dosage form. Some of preformulation parameters were performed to get the preliminary information about of procured Levofloxacin.

Physical Appearance

Physical appearance of drugs such as color, odor was analyzed by visual observation.

Purity and Identification

To check the purity and identification of the drug various studies have been performed.

UV Spectroscopy/Determination of Absorption Maxima (λ_{max})

To obtain structural information regarding chromophoric part of Levofloxacin, UV spectrophotometric method was used. Stock solution of 100 µg/mL of drug was freshly prepared in methanol and it was further diluted to obtain concentration of 50 µg/mL with methanol. Zero-order spectra were recorded in the range of 200–400 nm to determine the absorption maxima of Levofloxacin.

FTIR Spectroscopy

Infrared spectrum of any compound or drug gives information about the groups present in that particular compound. FTIR spectra were obtained using a FTIR spectrophotometer. The KBr disk method was employed by mixing small amount of drug powder with spectroscopic KBr and compressed in a vacuum press to obtain a disk. Infrared Spectrum was recorded by scanning over a wave number region of 400–4000 cm^{-1} using Nicolet omnic software. The characteristic I.R. spectrum and peaks were observed and were compared with the spectrum and peaks of the reference spectrum of the Levofloxacin.[22]

Melting Point

A capillary melting point apparatus was used to determine the melting point of the drug. A small amount of the drug was filled into the capillary previously sealed on one side and the melting point was analyzed on the melting point apparatus to observe the melting point range.

Solubility Analysis

The solubility study of the drug was carried out in different solvents in such as methanol, ethanol, acetic acid, chloroform, and PBS. Order to carry out these studies a minimum amount of solvent was selected and incremental quantities of drug were added into the solvent until the solution was saturated with the drug. After complete saturation, the solution was filtered and the concentration of the drug in the solution was estimated by measuring the UV absorbance of the solution and comparing it with the standard curve of the drug.

Partition Coefficient

Partition coefficient is a measure of drug lipophilicity and an indication of its ability to cross biomembarane factors. It is also useful in screening of biological properties. The partition coefficient is defined as ratio of unionized drug distributed between organic and aqueous phase. The partition coefficient of levofloxacin was determined in octanol: water. The octanol: water mixture was kept to equilibrate for 24 h in separating funnel. Then 10 mg of the Levofloxacin in 10 mL of water and 10 mL of n-octanol was added to it and after 24 h both the phase was separated and organic phase was analyzed using UV spectrophotometer. The concentration in the aqueous phase was determined by the difference between initial drugs added to mixture with drug concentration in the organic phase.

$$P_{O/W} = \text{Concentration in organic phase/Concentration in aqueous phase}$$

$$\text{Log Po/w} = \log (\text{Solute}_{octanol}/\text{Solute}_{water})$$

Development of Analytical Method by UV-Spectrophotometer and HPLC

Preparation of standard curve in methanol

Preparation of standard stock solution

Levofloxacin (10 mg) was accurately weighed and was taken in a clean and dry 100 mL volumetric flask and the volume was made with methanol up to 100 mL, to produce a stock solution of 100 µg/mL (stock A). The solution was scanned using UV spectroscopy at wavelength range of 200–400 nm.

Preparation of working solutions

From Stock A, different volumes were withdrawn separately in 10 mL volumetric flasks and volumes were made in each case made up to 10 ml with methanol to produce concentrations of 2–12 µg/mL. Absorbance values of these solutions were recorded at λ_{max} 298 nm against methanol as blank using UV-visible spectrophotometer (Shimadzu, Japan).

Preparation of standard curve in simulated tear fluid (STF pH 7.2)

Preparation of STF pH 7.2

STF were prepared as per simple dissolving of different chemicals, i.e., Sodium Chloride 6.7 g, Sodium Bicarbonate 0.20 g, and Calcium chloride 0.008 g volume was made upto 100mL.

Preparation of standard stock solution

Levofloxacin (10 mg) was accurately weighed and was taken in a clean and dry 100 ml volumetric flask and the volume was

made with STF (7.2) up to 100 mL, to produce a stock solution of 100 µg/mL and. The solution was scanned using UV spectroscopy at wavelength range of 200–400 nm.

Preparation of working solutions

From this solution 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL were withdrawn separately in 10 mL volumetric flasks and volume up to 10ml was made in each case made up to 10 ml with STF to produce concentrations of 2–12 µg/mL. Absorbance of these solutions was recorded at λ_{max} 298 nm against STF as blank using UV-visible spectrophotometer (Shimadzu, Japan).

Development of Analytical Method by HPLC

Equipment/chromatographic conditions

HPLC model	*Waters 2489*
Column	Nucleosil C18 (250×4.6×5 Mm)
Detector	UV detector
Flow rate	0.4 mL/min
Wavelength	298 nm
Mobile phase	Buffer: Acetonitrile (77: 23 v/v) (buffer: 20 mM KH_2PO4+1 mL triethylamine in 1 liter water, pH=2.5 adjust with orthophosphoric acid)

Preparation of standard solution

Levofloxacin (10 mg) was accurately weighed and was taken in a clean and dry 100 mL volumetric flask and the volume was made with prepared mobile phase up to 100 mL, to produce a stock solution of 100µg/mL. From this solution, 10 µg/mL solution was prepared by taking 1ml and volume was made up to 10 ml with the help of volumetric flask. The standard curve of levofloxacin was prepared by plotting a curve as peak area versus concentration of Levofloxacin solution in the mobile phase (298 nm) using varied concentrations of 5–60 µg/mL.[23,24]

Screening of excipients i.e. oil and smix (surfactant and cosurfactant mixture)

Selection of components (Oil, Surfactant, and Co-surfactant) was done on the basis of saturation solubility of drug in various as USFDA approval components. The components having the higher solubility were selected for the levofloxacin formulation.[25]

Results and Discussion

Preformulation Studies

Various preformulation parameters such as solubility, melting point, and partition coefficient were evaluated. FT infrared (FTIR) and UV spectroscopy were performed. Preformulation studies suggested that Levofloxacin was pure and free from impurities.

Purity and Identification Studies of Levofloxacin

Physical appearance

The Levofloxacin was visually observed and was found to be white powder, practically odorless as mentioned in I.P. shown in Figure 1.

Determination of λ_{max}

UV spectrum of Levofloxacin showed absorption maxima at 298 nm in methanol which is comparable with the reference spectrum of the drug 298 nm. The UV spectrum of Levofloxacin has been shown in Figure 2.

FTIR spectroscopy

An FTIR spectroscopy study was carried out to check the identity and purity of the obtained gift sample of drug levofloxacin. The spectrum obtained at wavelength from 4000 cm to 400 cm^{-1} is shown in Figure 3. FTIR of levofloxacin showed the following characteristic peaks at 3269.72 cm^{-1} due to carboxylic group, 2200 cm^{-1} due to alkanes group stretching, 1649 cm^{-1} due to stretching of carbonyl group, 1240.51 cm^{-1} due to stretching of amines, 1119.89 cm^{-1} due to the presence of halogen group. The results revealed the identity of the obtained gift sample. The spectrum is shown in Figure 3. The data are summarized in Table 1.

Melting point

The melting point determination was performed to check the purity of drug. The melting point of the Levofloxacin was found to the range between 225 and 227°C [Table 2], which complies with the standard i.e. 223–228°C. The drug melts completely over

Figure 1: Levofloxacin in powder form

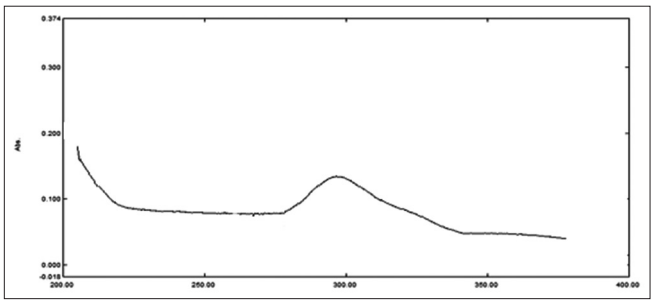

Figure 2: UV Visible spectrum of levofloxacin

Table 1: Reported and observed peaks of Levofloxacin

S. No.	*Observed frequency (cm^{-1})*	*Standard frequency (cm^{-1})*	*Interpretation*
1.	3269.72 cm^{-1}	3300–2500 cm^{-1}	-COOH
2.	2200 cm^{-1}	3000–2850 cm^{-1}	$-CH_3$
3.	1649cm^{-1}	1760–1690 cm^{-1}	C=O
4.	1240.51 cm^{-1}	1250–1000 cm^{-1}	C-N
5.	1119.89 cm^{-1}	1400–1000 cm^{-1}	F (Halogen group)

Table 2: Determination of melting point

Melting Point	*Standard*	*Test*
	225–227°C	223–228°C

a narrow temperature range indicating the crystalline pattern of the drug.

Solubility

The solubility of levofloxacin was studied by preparing saturated solution of the drug in different solvents. Saturated solution was prepared by dissolving the drug in specified volume of solvents until complete dissolution was observed. Solubility of levofloxacin in different solvents has been shown in Table 3 which is defined according to IP, 2018. Solubility parameter suggested levofloxacin is slightly soluble in water while highly soluble in methanol.

Partition coefficient

Partition coefficient is the ratio of concentration of a compound in a mixture of two immiscible phases at equilibrium. These coefficients are measure of the difference in solubility of the compound in these two phases, i.e., n-octanol/water. Concentration of levofloxacin in both phases was estimated and partition coefficient was calculated. Data obtained from partition coefficient determination suggest that Levofloxacin is lipophilic in nature [Table 4].

All these above observation in pre-formulation studies confirmed the identity and purity of the levofloxacin.

Development of Analytical Method by UV-Spectrophotometer and HPLC

Standard curve of levofloxacin in methanol

Standard curve of Levofloxacin was prepared in methanol and has been shown in Table 5 and Figure 4.

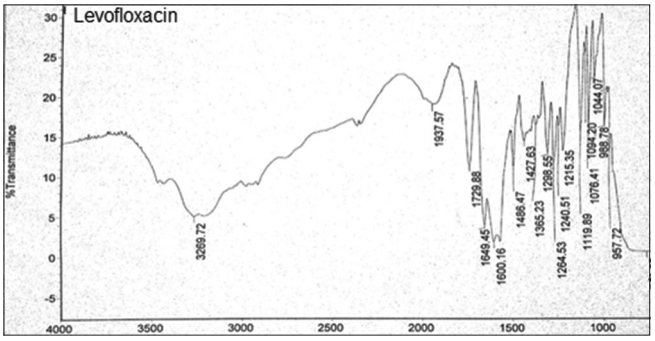

Figure 3: FTIR spectrum of levofloxacin

Table 3: Solubility of Levofloxacin in different solvents

Solvent	*Amount of drug*	*Parts of solvent required for one part of solute*	*Results*
Methanol	89 mg/ml	10–30	Soluble
DMSO	74 mg/ml	10–30	Soluble
Ethanol	26 mg/ml	1000–10000	Sparingly Soluble
Water	16 mg/ml	10–30	Soluble

Table 4: Partition coefficient of Levofloxacin

Reported Log P	*Observed Log P*
−0.39	−0.34±0.05

Values are reported as mean±S.D., *n*=3

Preparation of standard curve in STF 7.2

Standard curve of Levofloxacin was prepared in STF 7.2. Results are shown in Table 6 and Figure 5.

Development of Analytical Method by HPLC

Analytical method of Levofloxacin was developed as per described method in section materials and methods with HPLC and shown in Figure 6.

The standard curve of levofloxacin was prepared by plotting a curve as peak area *vs* concentration of levofloxacin solution in the mobile phase (298 nm) using varied concentrations of 5–60 µg/mL. The plot showed good linearity in the working concentration range. The correlation coefficient value and the linear equation obtained revealed the linearity in the developed method. The obtained data are presented in Table 7 and Figure 7.

Screening of excipients i.e. oil and smix (surfactant and cosurfactant mixture)

The solubility of levofloxacin was evaluated in different oils, i.e., castor oil, olive oil, ethyl oleate, and isopropyl myristate visually only. Ethyl oleate was found to be highly suitable for us due to its high miscibility with levofloxacin as well as it is reported to be safe and biocompatible for ocular drug delivery.

Tween 80 was selected as a surfactant because of its desired properties of making stable o/w Nanoemulsion. Tween 80 can be used for ocular purpose in concentration of upto 40% without producing any toxicity.

Table 5: Calibration curve of Levofloxacin in methanol

S. No.	*Concentration (µg/ml)*	*Absorbance*
1.	2	0.29
2.	4	0.36
3.	6	0.47
4.	8	0.58
5.	10	0.66
6.	12	0.76

Table 6: Calibration curve of Levofloxacin in STF 7.2

S. No.	*Concentration (µg/ml)*	*Absorbance*
1.	2	0.16
2.	4	0.31
3.	6	0.42
4.	8	0.53
5.	10	0.63
6.	12	0.74

STF: Simulated tear fluid

Table 7: Calibration curve of levofloxacin using HPLC method

Concentration (µg/mL)	*Peak Area*
5	27779
10	117129
15	194782
20	306301
25	384836
30	474072
40	652544
50	833356
60	997788

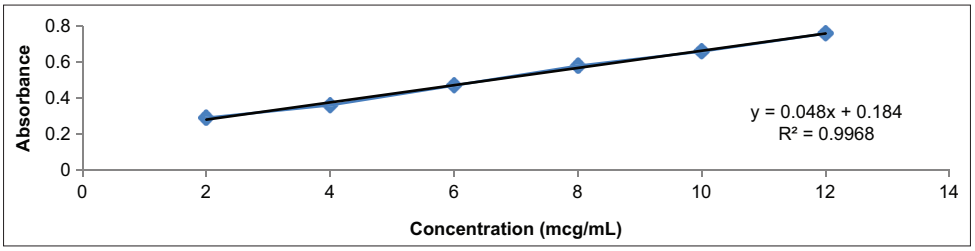

Figure 4: Standard plot of Levofloxacin in methanol

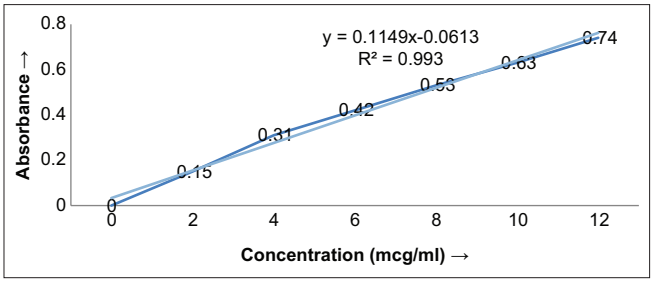

Figure 5: Standard plot of levofloxacin in simulated tear fluid (STF 7.2)

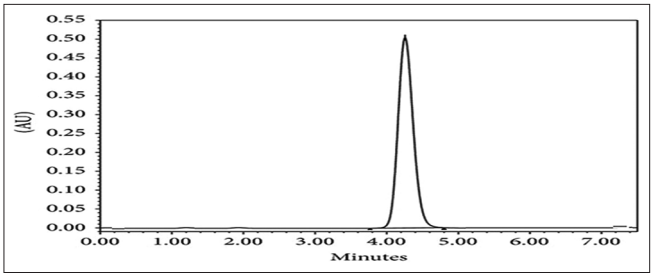

Figure 6: HPLC chromatogram of levofloxacin

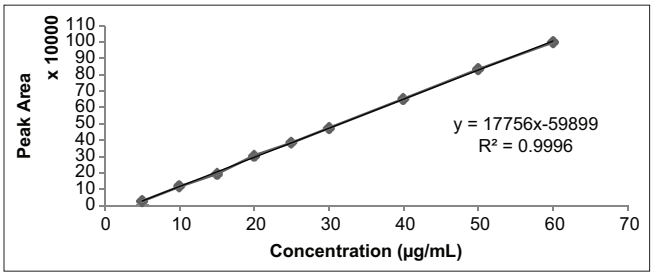

Figure 7: Calibration curve of levofloxacin using HPLC method

Soya lecithin was selected as a co-surfactant as because it is biocompatible and non-immunogenic amphiphilic molecules having composition similar to biomembrane. Moreover, it imparts a negative charge prepared colloidal system thus in our purposed study negatively charged globules has to be developed to that in late stages it can be coated with cationic mucoadhesive polymers.

Conclusion

The preformulation parameter such as melting point, UV spectrophotometry analysis, solubility profile, partition coefficient, and compatibility studies by FTIR make the most of the probability of getting a formulation that is efficacious, safe, and stable product and at the same time give optimization of the drug product quality. On the basis of these studies, it was finished that the Levofloxacin serves as appropriate candidate for nanoemulsion for opthalmic use.

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