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Ocular Drug Delivery of Levofloxacin Loaded Chitosan Nanoparticle by Emulsion Solvent Diffusion Method

Nishanth Kumar*[1], S Parthiban[2], GP Senthil Kumar[3]
T Tamizh Mani[4]
[1*]Bharathi College of Pharmacy Department of Pharmaceutics Bharathi nagara-571422
Maddur Taluk, Mandya District, Karnataka, India

Abstract:

Aim: *The aim of the present study is an attempt to formulate and evaluate Levofloxacin loaded chitosan nanoparticles for the sustained ocular drug delivery.*

Methods*: Levofloxacin loaded chitosan nanoparticle was prepared by emulsion solvent diffusion method using chitosan as polymer. The effect of various processing parameters, such as drug: polymer ratio, and entrapment efficiency, zeta potential, in vitro drug release, kinetic studies and stability studies.*

Results: *FT-IR study revealed that there was no interaction between the drug (Levofloxacin) and polymer (chitosan). The highest % entrapment efficiency is for F4 92.64% and the highest percent of drug content F4 93.25%. Thus the results showed that increase in polymer ratio, increases the entrapment efficiency and percent drug content also increases in the formulations F1-F4. The shape and surface morphology of optimized CS-NP formulation was studied by SEM. The microphotographs reveal that the particles are uniform in size and roughly spherical in shape. The zeta potential was measured for the chitosan nanoparticle formulations were found between 20.5 mV, -0.857mV which are suitable formulations were sufficient to keep the particles stable. In-vitro release study of Levofloxacin from various formulations was conducted for 24 hrs by using dialysis membrane. All the formulation showed more than 20 % in the first 1 hr due to the presence of un-entrapped drug and the drug entrapped on the surface of polymer core which released faster showing dose dumping which is suitable to produce the initial effect of drug.*

Conclusion: *The best fit release kinetic was achived with diffusion followed by Higuchi plot.it was found that all formulation follows Higuchi model. The 'n' values for all the formulation were found to be more than 0.5. This indicates that the release approximates Non-Fickian diffusion mechanism. this chitosan nanoparticle opens an new prospective sustained release against bacterials for ocular drug delivery.*

Key Words*: Nanoparticle, Chitosan, Levofloxacin, Emulsion solvent diffusion method.*

1.INTRODUCTION:

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. 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. Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy. Potent immune suppressant therapy in transplant patients and the developing epidemic of acquired immune deficiency syndrome have generated an entirely new population of patients suffering virulent uveitis and retinopathies. Conventional ophthalmic solution, suspension, and ointment dosage forms no longer constitute optimal therapy for these indications. Research and development efforts to design better therapeutic systems particularly targeted to posterior segment are the primary focus of this text[1].

Drainage of an administered drug dose by the nasolacrimal system can occur when the volume of fluid in the eye exceeds the normal lachrymal volume of about 7-10 µl. In contrast, The application of one to two drops of a drug medication applied by an eye-dropper as the drug delivery device represents roughly 50–100 µl. Much of this dose is wasted or rapidly drained. The remaining applied drug solution is diluted by induced increased lachrymation and physiological

tear turnover produced by the applied drug solution[2].

Direct application delivers high concentrations of antimicrobial agents to the surface of the eye conveniently, quickly and with minimal systemic exposure to the agent. However, antibacterials are rapidly dissipated from the tear film and intraocular penetration of topical antibacterial agents is generally poor, necessitating intensive application for successful treatment of corneal infections. Therapeutic concentrations are rarely achieved at other sites in the eye[15]. The major goals in designing nanoparticles as a delivery system are to control particle size, Surface properties and release of pharmacologically active agents so as to achieve the site specific action of the drug at the rationale rate and dose. Polymeric nanoparticles offer some specific advantages over liposomes. For instance, They help to increase the stability of drugs/proteins and possess useful controlled release properties[3].

The nanoparticle are especially useful in ocular drug delivery as they can improve the corneal absorption of drugs and progress the ocular bioavailability of both hydrophilic and lipophilic drugs. Biocompatible and mucoadhesive properties of nanoparticle improve interaction with ocular mucosa[4].

According to a working group of the European Science Foundation in 2004, "Nanomedicine" is built on complex systems of nanometer-scale size consisting of at least two components, one of which is an active pharmacological ingredient and the whole system leading to a special function related to the diagnosis, treatment, or prevention of disease. In this context, nano-scale is taken to include active components or objects in the size range from 1 to 100 of nanometers. Nanomedicines in the form of drug carriers (*e.g.*, particles, liposomes, dendrimers, *etc.*) play an important role to warrant safe and efficient delivery of active compounds to their intended site of action. The added advantages of nanoparticles over microparticles include the ability to improve drug encapsulation, Pharmacokinetics, Bioavailability as well as therapeutic efficacy. Nanotechnology has opened up a new era in the field of drug delivery. For nanoparticulate drug delivery, the polymeric carriers should be easy to synthesize and characterize inexpensive, biocompatible, biodegradable, non-immunogenic, non-toxic and water soluble[5,6].

2. MATERIALS AND METHODS:

Levofloxacin is a gift sample from the Micro Laboratories ltd. , Bangalore. Chitosan was obtained from shreeji chemicals Mumbai-400 002. (India). Acetic acid, Polyvinyl alcohol and acetone, poloxamer188 are purchased from SDFCL s d fine-chem ltd industrial estate. 248, worli road Mumbai-30. chloroform are purchased from Fisher scientific. All the reagents and solvents used were of analytical grade satisfying pharmacopoieal standards.

2.1 Preparation of chitosan nanoparticle[7]:

Levofloxacin loaded chitosan nanoparticle were prepared by emulsion solvent diffusion method, This method is based on the partial miscibility of an organic solvent with water. Levofloxacin, acetic acid were dissolved in 5ml mixture of chloroform and acetone (4:1). An o/w emulsion is obtained upon injection an organic phase into chitosan solution containing a stabilizing agent (Poloxamer) under mechanical stirring, followed by high pressure homogenization. The emulsion is then diluted with a large amount of water to overcome organic solvent miscibility in water. Polymer precipitation occurs as a result of the diffusion of organic solvent into water, leading to the formation of nanoparticles.

Table. 1-Formulation design of Chitosan nanoparticles.

Ingredients in % w/v	Drug	polymer	Acetic acid	Polyvinyl alcohol	Poloxamer188	Chloroform (ml)	Acetone(ml)	Purified water(ml)
F1	0.5	0.25	0.5	1	0.5	4	1	100
F2	0.5	0.5	0.5	1	0.5	4	1	100
F3	0.5	0.75	0.5	1	0.5	4	1	100
F4	0.5	1.0	0.5	1	0.5	4	1	100

2.2 Characterization of prepared nanoparticle.

2.2.1 Drug-Polymer compatibility studies by FT-IR

The FT-IR spectra of pure drug Levofloxacin(A), chitosan(B) and Physical mixture of drug polymer(C) were recorded to check drug polymer interaction and stability of drug (Fig1)

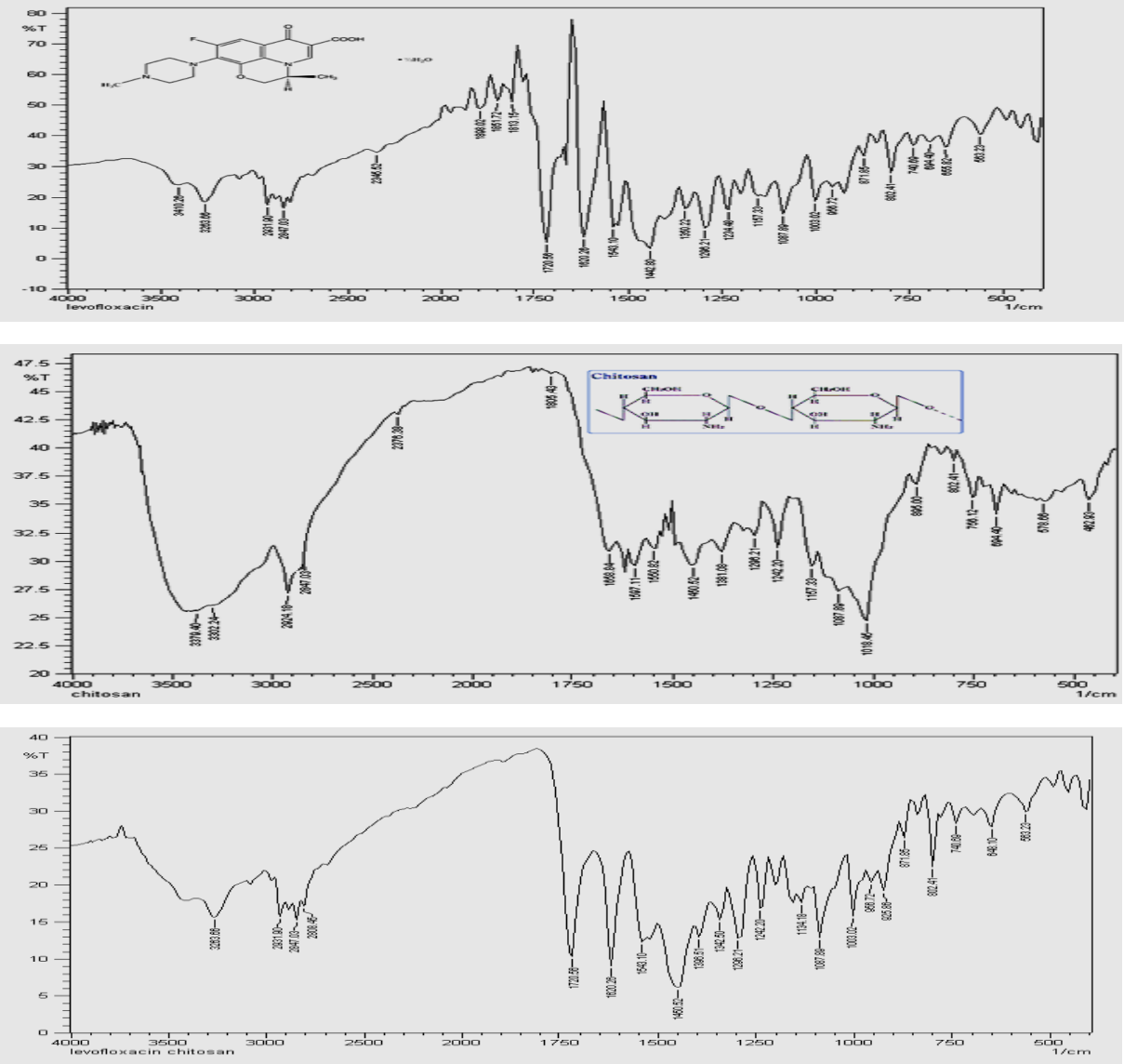

Fig. 1- FT-IR spectra of (A)Pure Levofloxacin, (B)Chitosan, (C)Physical mixture of drug polymer

2.2.2 Drug content and entrapment efficiency:

Table. 2- Percent drug content and entrapment efficiency

Sr.No	Formulation code	% Entrapment efficiency±SD	% Drug Content±SD
1	F1	58.86±0.40	74.25±0.19
2	F2	69.34±0.28	78.23±0.27
3	F3	78.23±0.50	89.24±0.28
4	F4	92.64±0.37	93.25±0.23

A. Percent Drug content:

Total drug content in the Levofloxacin loaded chitosan nanoparticle formulation was determined by dissolving Levofloxacin loaded chitosan nanoparticle 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 λ max 288 nm against blank.

B. Entrapment efficiency:

The entrapment efficiency (EE %) of Levofloxacin loaded chitosan nanoparticle was determined by

centrifugation method. 2ml of nano-suspension 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. The % entrapment was determined by following formula:

$$\text{Percentage entrapment} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug added}} \text{X100}$$

Amount of drug present in supernatant and sediment gave a total amount of drug present in system.

2.2.3 Scanning electron microscopy (SEM):

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 100Polaron 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.

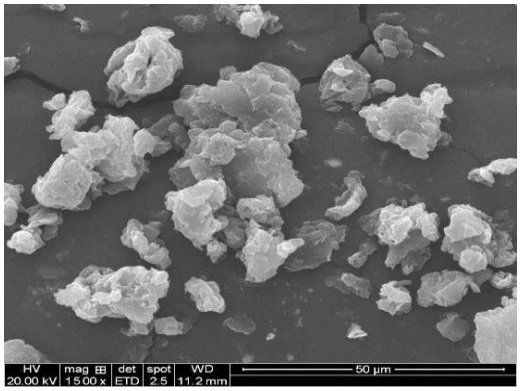

Fig. 2: SEM of formulation F3

2.2.4 Zeta potential

Zeta potential of the Chitosan nanoparticle was measured by Malvern zetasizer. For zeta potential measurements this light splits to provide an incident and reference beam. The incident laser beam passes through the centre of the sample cell, and the scattered light at an angle of about 130 is detected. When an electric field is applied to the cell, any particles moving through the measurement volume will cause the intensity of light detected to fluctuate with a frequency proportional to the particle speed and this information is passed to the digital signal possessor and then to a computer. Zetasizer software produces a frequency spectrum from which the electrophoretic mobility hence the zeta potentials calculated.

2.2.5 *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 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).

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 20 % in the first 1 hr due to the presence of un-entrapped drug and the drug entrapped on the surface of polymer core which released faster showing dose dumping which is suitable to produce the initial effect of drug. It has been found that from the CS-NP formulation, the release were F1- 95.28%, F2- 88.14 %, F3- 78.26% and F4 - 70.81%. The increase in polymer ratio from F1 to F4 causes decrease in the drug release and the release was more controlled by increasing the polymer ratio.

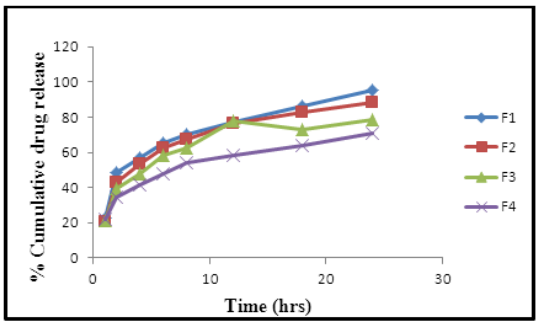

Fig. 3- % cumulative drug release of chitosan nanoparticle

2.2.6 Kinetic modeling:

Upon the application of different drug release model kinetics is given in Table no 3. In order to understand the kinetic and mechanisms of drug release, the result of in vitro drug release study of chitosan nanoparticle were fitted with various kinetic equation like zero order (cumulative % release vs time), first order (log % drug remaining vs time), Higuchis model (cumulative % drug release vs square root of time), peppas plot (log of cumulative % drug release vs log time). R^2 and n

values were calculated for the linear curve obtained by regression analysis of the plot. it was found that all formulation follows Higuchi model. The 'n' values for all the formulation were found to be more than 0.5. This indicates that the release approximates Non-Fickian diffusion mechanism. (Table. 3)

Table. 3- Correlation coefficients according to different kinetic equations.

Formulation code	Zero order	First order	Higuchi Matrix	Peppas plot	' *n* ' value	Best Fit Model
F1	0.804	0.961	0.926	0.5312	0.8828	HIGUCHI
F2	0.7792	0.936	0.9253	0.5479	0.8872	HIGUCHI
F3	0.7139	0.794	0.8943	0.5363	0.8599	HIGUCHI
F4	0.8549	0.8801	0.9381	0.5155	0.8056	HIGUCHI

2.2.7 Stability studies[8-10]:

The accelerated stability studies for formulations were performed for 6 months according to the ICH guide lines. Drug entrapment and drug release were fixed as physical parameters for stability testing and the results were given in the table 4

Table no. 4 -Accelerated Stability studies for the formulations

Temperature And RH	% Drug Entrapment After (months)				% Drug Release After (months)			
	0	1	3	6	0	1	3	6
40±2 ℃and 75±5% RH	95.74 ± 0.24	94.89 ± 0.38	92.23 ± 069	90.48 ± 019	85.26	85.26	83.26	80.48

3. RESULTS AND DISCUSSION:

Chitosan nanoparticles containing Levofloxacin were successfully prepared by emulsion solvent diffusion method. FT-IR study revealed that there was no interaction between the drug (Levofloxacin) and polymer (chitosan). The % entrapment efficiency F1 58.86%, F2 69.34%, F3 78.23%, F4 92.64% and the percent of drug content are as fallows F1 74.25%, F2 78.23%, F3 89.24%, F4 93.25%. Thus the results showed that increase in polymer ratio, increases the entrapment efficiency and percent drug content also increases in the formulations F1-F4. The shape and surface morphology of optimized CS-NP formulation was studied by SEM. The microphotographs reveal that the particles are uniform in size and roughly spherical in shape. The presence of aggregates might be attributed to a short redispersion time after centrifugation and drying at room temperature. The zeta potential was measured for the chitosan nanoparticle formulations were found between 20.5 mV, -0.857mV which are suitable formulations were sufficient to keep the particles stable. *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 20 % in the first 1 hr due to the presence of un-entrapped drug and the drug entrapped on the surface of polymer core which released faster showing dose dumping which is suitable to produce the initial effect of drug. It has been found that from the CS-NP formulation, the release were F1- 95.28%, F2- 88.14 %, F3- 78.26% and F4 - 70.81%. The increase in polymer ratio from F1 to F4 causes decrease in the drug release and the release was more controlled by increasing the polymer ratio. it was found that all formulation follows Higuchi model. The 'n' values for all the formulation were found to be more than 0.5. This indicates that the release approximates Non-Fickian diffusion mechanism. **References:**

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