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Evaluation of Anti Bacterial Efficacy of Chitosan Loaded Levofloxacin Nanoparticle Prepared By Emulsion Solvent Diffusion Method

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ABSTRACT

The aim of the present study was an attempt to formulate and evaluate Levofloxacin loaded chitosan nanoparticles (CS-NP) by Emulsion solvent diffusion method using poloxamer 188 as surfactant; about eight different formulations (F1-F8) were prepared by changing the ratios of drug and excipients . Among all the formulations F3 was selected as optimized formulations based on the physico chemical parameters and drug release studies and it was incorporated in to 1% W/W of Carbopol gel (GF3) to obtained ophthalmic gel ,further, it was evaluated for antimicrobial activity against *S. aureus* and *Bacillus subtilis*. Compatibility studies by FT-IR showed no significant interactions between drug and excipients. The prepared Chitosan nanoparticle were characterized for different parameters like particle size analysis, zeta measurement, SEM, % drug content, entrapment efficiency, FT-IR, DSC, *In-vitro* release. The Gel containing chitosan nanoparticles (GF3) were evaluated for different parameters like physical examination, pH, spread ability, viscosity, reological property, % drug content and *In-vitro* release. The *in vitro* dug release and antimicrobial efficacy of GF3 formulation was compared with marketed product. From the results it was observed that the formulation controlled the drug release over a period of 24 hrs following Higuchi model and nonfickian diffusion mechanism with better antimicrobial action than the marketed product. The ocular irritancy study confirmed that ther is no irritation to the eye. From the above study we can conclude that the Levofloxacin loaded CS-NP were successfully prepared by emulsion solvent diffusion method and it is found to be suitable for sustained ocular drug delivery having improved antibacterial action.

Keywords: Levofloxacin, Chitosan nanoparticles (CS-NP), DSC, FT-IR and Antimicrobial studies.

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INTRODUCTION

Despite eyes are among the most readily accessible organs in the body, good ocular bioavailability is still a challenging task. The bioavailability of ocular drugs in conventional system that is aqueous solution is usually low because of quick elimination from the eye reflex blinking and tear drainage. Corneal barrier also plays a significant role in low ocular bioavailability. Significant efforts have been made over decades to improve their ocular bioavailability of administered drug that is inserts collagen shield and colloidal system such as liposome's, nanoparticles and nanocapsules. Among all, nanoparticles come out to be the most promising application in ocular drug delivery. Treatment with nanoparticles system increases bioavailability, reduces administration frequency and promotes drug targeting [1].

Nanoparticles are colloidal carriers with a size range of 10 to 1000 nm. For ophthalmic delivery, nanoparticles are generally composed of lipids, proteins, natural or synthetic polymers such as albumin, sodium alginate, chitosan, (PLGA), (PLA) and polycaprolactone[2].

Nanoparticles represents a promising candidate for ocular drug delivery because of small size leading to low irritation and sustained release property avoiding frequent administration. However, like aqueous solutions, nanoparticles may be eliminated rapidly from precorneal pocket. Hence, for topical administration nanoparticles with mucoadhesive properties have been developed to improve precorneal residence time. chitosan and hyaluronic acid are commonly employed to improve precorneal residence time of nanoparticles. Chitosan coating is most widely explored for improving precorneal residence of nanoparticles. The chitosan is positively charged and hence it binds to negatively charged corneal surface and thereby improves precorneal residence and decreases clearance[3, 4].

The potential of chitosan nanoparticles for ocular drug delivery and their interactions with ocular mucosa *in vivo* and also toxicity in conjunctival cell cultures was studied and it was reported that the chitosan nanoparticles are able to interact and remain associated to the ocular mucosa for extended periods of time, thus being promising carriers for enhancing and controlling the release of drugs to the ocular surface [5, 6].

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. Fluoroquinolones eye drops have been the most often recommended treatment of bacterial conjunctivitis. Levofloxacin a third generation fluoroquinone antibiotic shows good activity against staphylococcus aureus on cornea and conjunctiva. The dosage regimen includes

one drop in every 1-2 hrs for 3 days and then in every 4-5 hrs. Hydrophobic drugs are difficult to dispense as eye drops as these drugs shows poor ocular bioavailability compare to hydrophilic drugs[7, 8,9,10].

However, literature search indicates that most of Chitosan nanoparticles has not been studied in detail other than ionic gelation method and hence in the present study is attempted to demonstrate the influence of Levofloxacin concentration on the physicochemical characteristics and release profile of the Chitosan nanoparticles prepared by emulsion solvent diffusion method for the improved ocular activity.

The specific objective of the study is to formulate and evaluate the Levofloxacin loaded Chitosan based polymeric nanoparticle gel by emulsion solvent diffusion method to retain the dosage form in the ocular region, to improve the bioavailability, reduce dose frequency, toxicity and patient compliance.

MATERIALS AND METHOD

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, acetone, poloxamer188 are purchased from SDFCL S D fine-chem LTD industrial estate. chloroform are purchased from Fisher scientific. All the reagents and solvents used were of analytical grade satisfying pharmacopoieal standards.

Drug-Polymer compatibility studies by FT-IR

To determine the drug-Polymer compatibility, FT –IR studies were carried out. The IR spectra of pure drug (Levofloxacin hemihydrate), Chitosan, and their Physical mixture (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.

METHODS

Preparation of chitosan nanoparticle

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, further the optimized formulation were incorporated in to 1 % w/w of Carbopol gel which was prepared by cold mechanical method and used for further studies [11].

The composition and formulation design of these chitosan nanoparticle systems is demonstrated in Table 1.

Table.1: Formulation Design for the Preparation of Levofloxacin loaded chitosan nanoparticle

Ingredients in % w/v	F1	F2	F3	F4	F5	F6	F7	F8
Levofloxacin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chitosan	0.25	0.5	0.75	1.0	0.5	0.5	0.5	0.5
Acetic acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Polyvinyl alcohol	1	1	1	1	1	1	1	1
Poloxamer188	0.5	0.5	0.5	0.5	1.0	1.5	2.0	3.0
Chloroform(ml)	4	4	4	4	4	4	4	4
Acetone(ml)	1	1	1	1	1	1	1	1
purified water(ml)	100	100	100	100	100	100	100	100

CHARACTERIZATION OF CHITOSAN NANOPARTICLE[12,13,14]

Particle size analysis and Zeta potential measurement

The size and zeta potential of CS-NP 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 sample 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 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.

Percent Drug content

Total drug content 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.

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. Thus the amount of free drug was calculated.

% entrapment = Total drug – free drug/ Total drug x 100

FT-IR Studies for optimized formulation

To determine the drug-Polymer encapsulation for the optimized formulation FT –IR studies were carried out. The spectra were recorded by using the potassium bromide (KBr) disk technique. FT-IR measurement over the range of 4000 – 600 cm^{-1} was performed.

DSC investigation of optimized formulation

Drug encapsulation study was also performed by Differential Scanning Calorimetry (DSC). Thermal characteristics of the optimized were performed by using an automatic thermal analyzer system (Mettler DSC 823, Germany). The entire samples were run at a scanning rate of 10 °C per min from 25 - 300 °C.

***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).

Evaluation parameters for the Chitosan nanoparticle gel

The optimized formulation F3 can be incorporated into carbopol gel to obtained ophthalmic gel(GF3).

Physical examination

Macroscopic examination for visual appearance, color, 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 gm 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 Chitosan nanoparticle 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.

Percent drug content

The drug content was determined by 2 gm Chitosan nanoparticle 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 λ max 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 Chitosan nanoparticle 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.[7] All procedure describe were reviewed and approved by Institutional of animal ethical committee (IAEC) of Bharathi college of pharmacy BCP/IAEC/PCEU/04/2015.

Antimicrobial efficacy studies

The antimicrobial efficacy studies were carried out by disc diffusion technique. Optimized formulation are screened for their *in vitro* anti-microbial activity against *Staphylococcus aureus* and *Bacillus subtilis* are compared with pure drug , standard drug Amikacin (30µg) and marketed eye drops (Levobact-0.5%, B. No- LFAH0011). The antibacterial activities were carried out by disc diffusion method. The concentrations of the pure drug, optimized formulation and marketed eye drops were taken in DMSO and used in the concentration of 20 µg and 30 µg /disc.

The target microorganisms were cultured in Mueller–Hinton broth (MHB). After 24 hrs the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strain. Disc made of Whatman No.1, diameter 6 mm was pre-sterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile disc papers. Then the prepared discs were placed on the culture medium. Standard drug Amikacin (30µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37 ºC for 24 hrs. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-microbial activity[8].

Intermediate Stability studies

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.[9-11].

RESULTS AND DISCUSSION

Compatibility studies

FTIR studies to find out the compatibility of drug with excipients

The FT-IR spectra of pure drug (Levofloxacin hemihydrate), Chitosan, and their Physical mixture (1:1) given in the Figure No.1 and Figure No.2 respectively. The IR spectra of pure drug shows principle peaks at 1720.56 cm^{-1}(C=O Stretching vibration of –COOH group), 1296.21 cm^{-1} (C-N stretching), 1087.89 cm^{-1} C-F (Stretching). The physical mixture on the other hand shows peaks at

1720.56, 1296.21 and 1087.89cm^{-1}. From these we have concluded that the physical mixture of drug, Levofloxacin does not show any major interaction with the formulation.

Drug content and entrapment efficiency

The percentage of drug content and entrapment efficiency of Levofloxacin in different CS-NP formulations with different drug, polymer and surfactant ratio was found to spectrophotometrically. The highest drug content was observed in F8 (98.26±0.31) because of high surfactant ratio. Highest entrapment efficiency was observed in F7 and F8 with 90.21% and 94.74% respectively. The high drug entrapment may be observed due to the presence of high Poloxamer ratio as surfactant and it was observed that increase in the entrapment efficiency. Highest entrapment of 94.74% was found in F8 (drug: polymer: surfactant in the ratio of 0.5:0.5:3) and lowest entrapment of 58.86% was found in F1 due to low polymer ratio (drug: polymer: surfactant in the ratio of 0.5:0.25:0.5).

The results showed that increase in polymer ratio, increases the entrapment efficiency and percent drug content also increases in the formulations F1-F4. And the ratio of surfactant is increased from the F5-F6 also showed the increase in the entrapment efficiency and the present of drug content.

FT- IR studies for optimized formulation

FTIR studies for optimized formulation F3 was carried out and from spectra, we have observed that the absence of characteristic peaks of pure drug which indicates the drug was encapsulated in the nanoparticles shown in Figure 3.

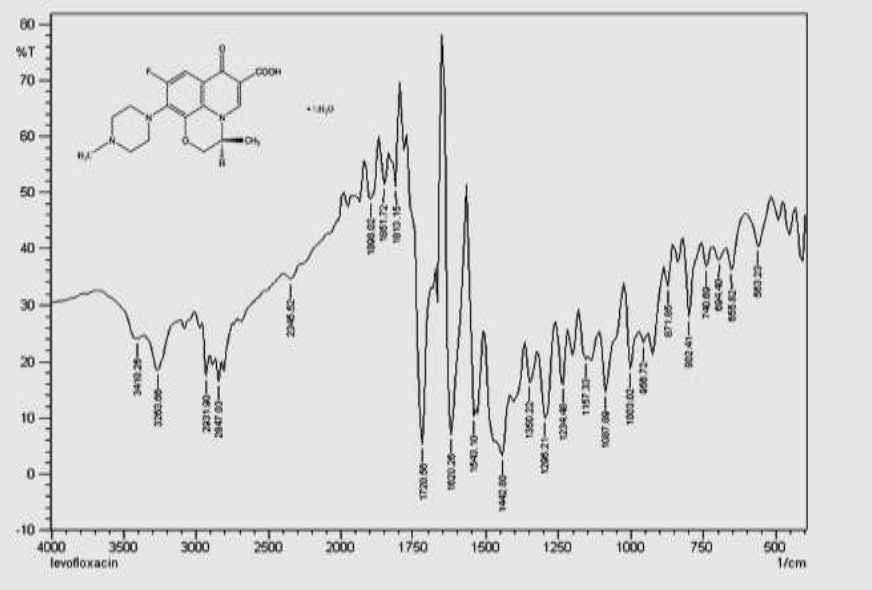

Figure.1: FTIR spectrum of Levofloxacin

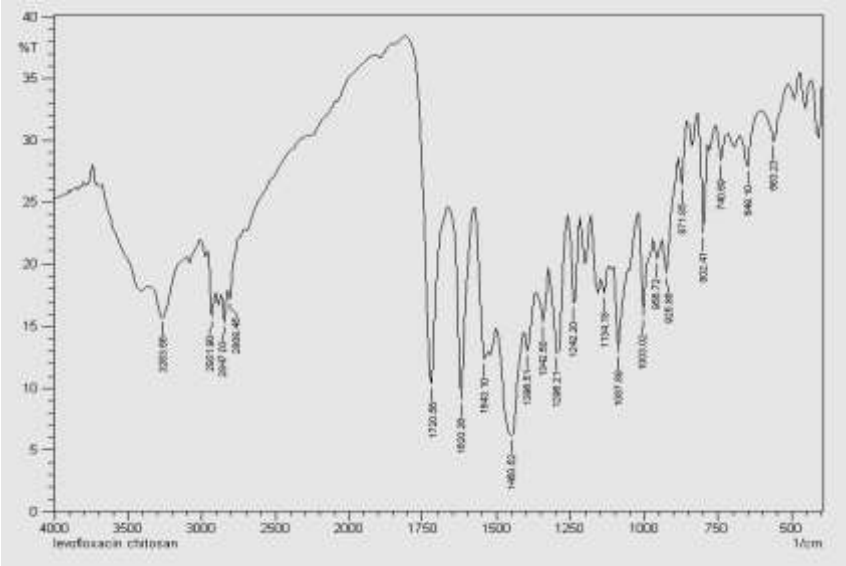

Figure 2: FTIR spectrum of physical mixture of Levofloxacin and Chitosan

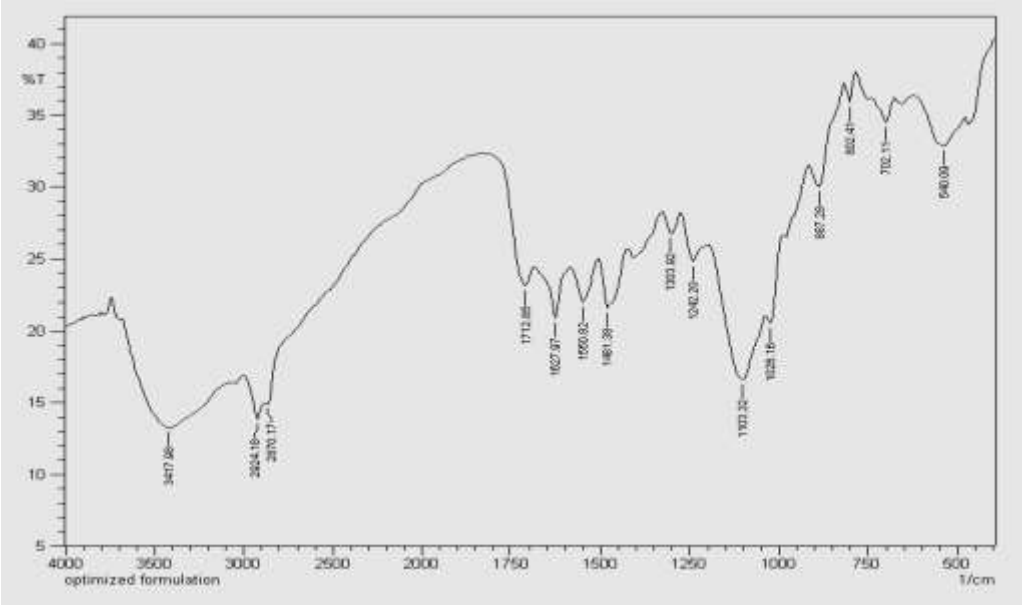

Figure.3: FTIR spectra of optimized formulation F3

DSC investigations of optimized formulation

DSC investigation was carried out for the optimized formulation F3 and compared with pure drug. The thermo gram obtained was shown in (Figure 4, 5). The absence of endothermic peak of pure drug in the optimized formulation confirmed that the drug was fully encapsulated in the prepared chitosan nano particle

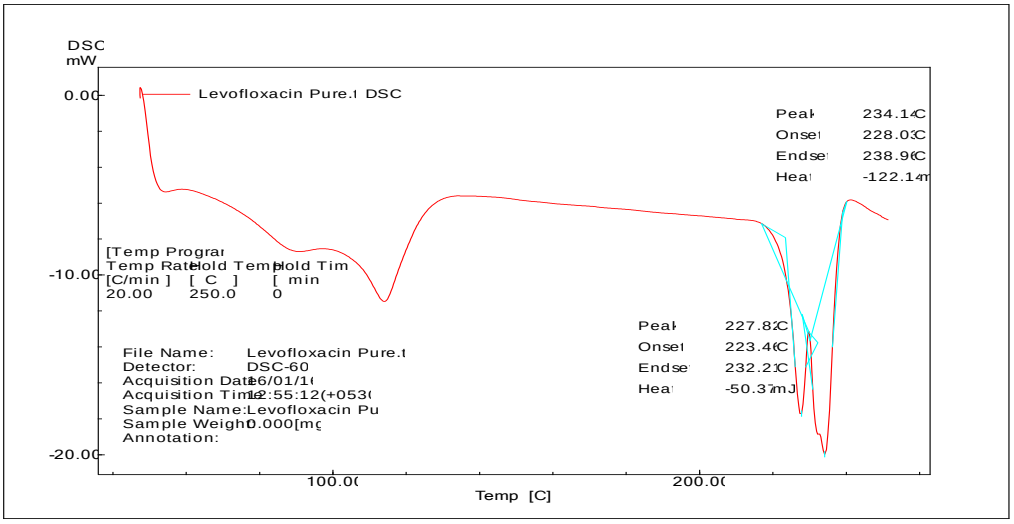

Figure 4: DSC thermo graph of pure drug Levofloxacin hemihydrate

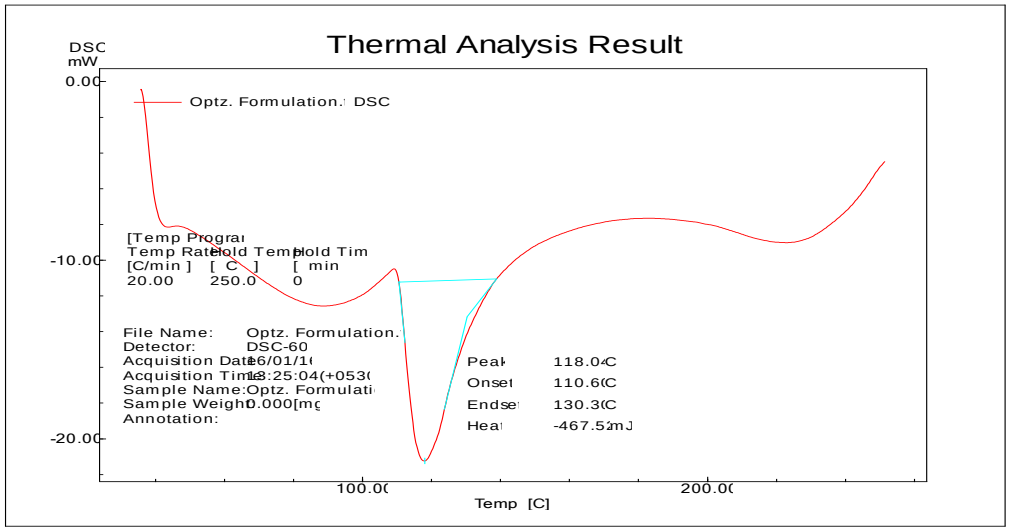

Figure 5: DSC thermo graph of optimized formulation

Particle size analysis:

Particle size of the Chitosan nanoparticle was analyzed by using Malvern particle size analyzer for the optimized formulations F3 as shown in Figure 6. The mean particle size of optimized formulation F3 was found to be 95.57 nm with particle size distribution less than 1µm.

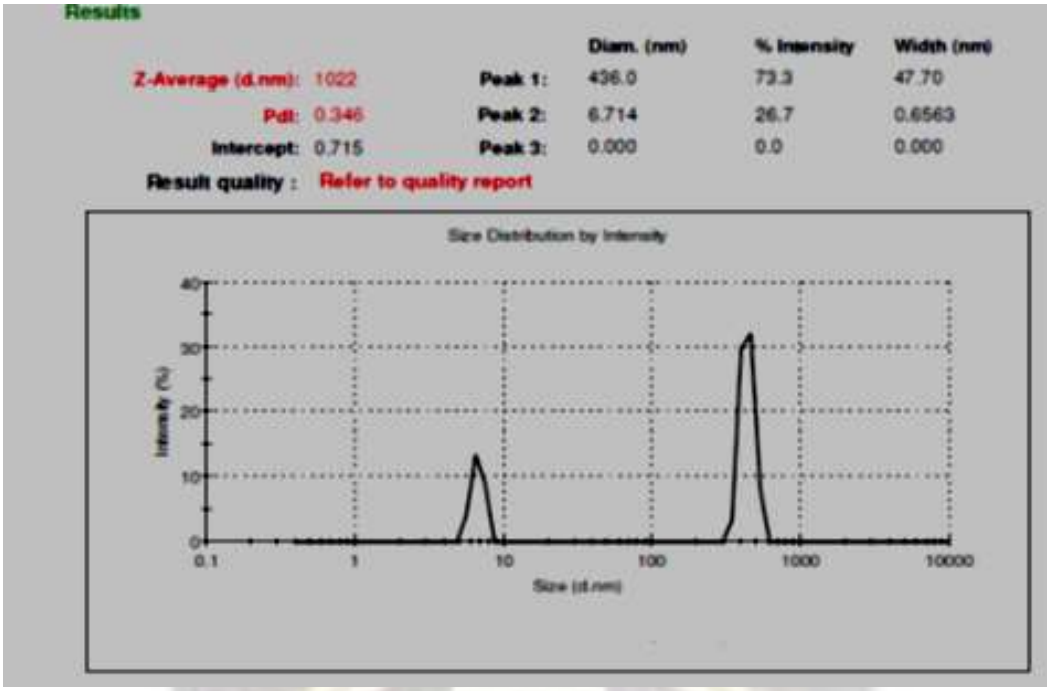

Figure 6: Particle size distribution analysis of formulation F3

Zeta potential measurement

Zeta potential is a key factor for evaluation of the stability of colloidal dispersion. It was currently admitted that zeta potentials were required for full electrostatic stabilization. The zeta potential was measured for the optimized formulation F3. The values of zeta potential of CS-NP formulations F3 were found 20.5 mV(Figure 7). From the results we have observed that the formulations were sufficient to keep the particles stable.

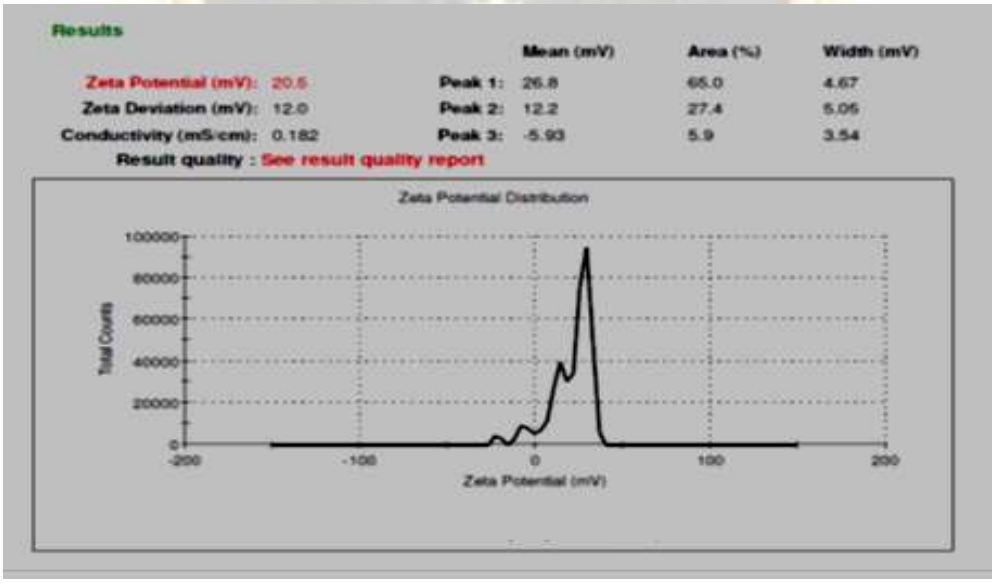

Figure7: Zeta potential analysis of formulation F3

Scanning electron Microscopy

The shape and surface morphology of optimized CS-NP formulation (F3) was studied by SEM. The microphotographs reveal that the particles are uniform in size and roughly spherical in shape (Figure 8). The presence of aggregates might be attributed to a short redispersion time after centrifugation and drying at room temperature.

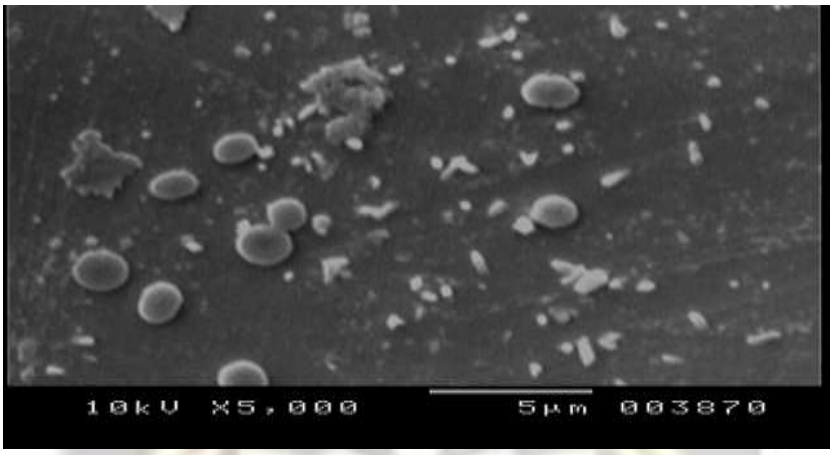

Figure 8: SEM of the optimized formulation F3

***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 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.

We observed that the drug release in the F5 (79.54%) was reduced in the F6 (75.89%), due to increased ratio of poloxamer. As the poloxamer ratio is increased the drug release will reduces as in case of F7 (70.46%) and in F8 (66.45). The results are shown in Figure 9, 10.

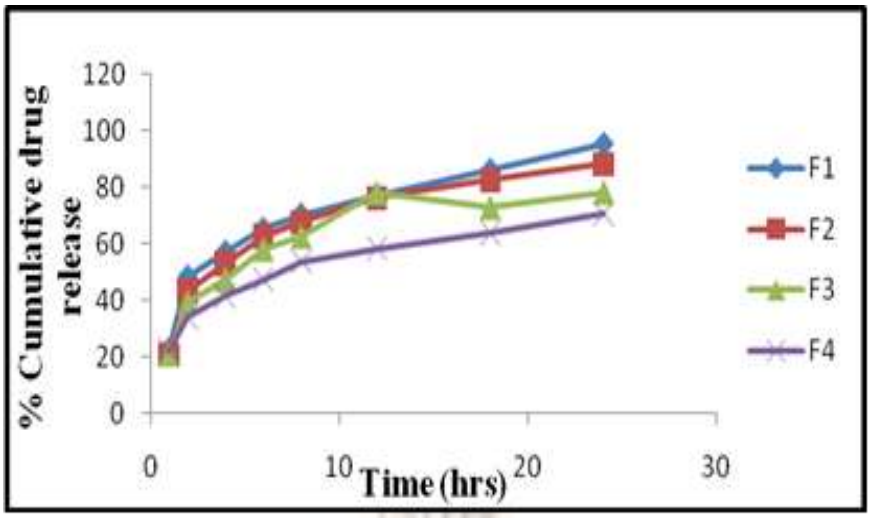

Figure 9:*In-vitro* release profile of formulations F1–F4 in phosphate buffer pH 7.4

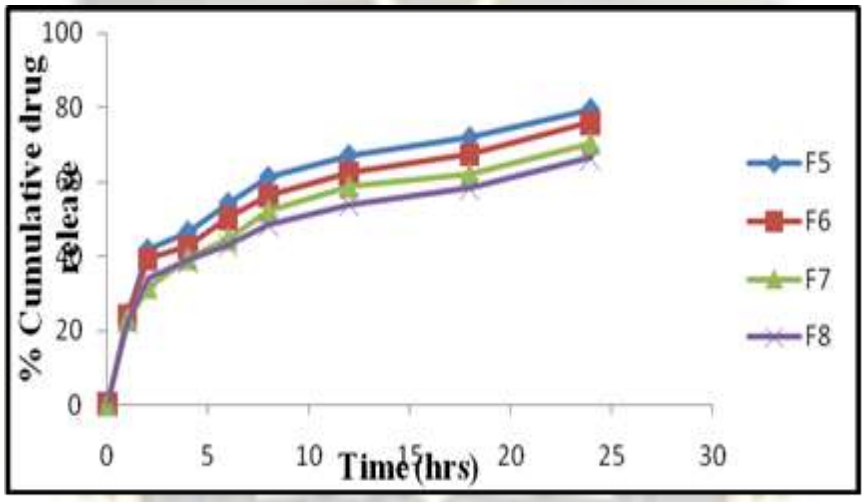

Figure 10: *In-vitro* release profile of formulations F5 –F8 in phosphate buffer pH 7.4

Kinetic model data analysis

Upon the application of different drug release model kinetics, 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.

Preparation and Evaluation of Levofloxacin loaded chitosan nanoparticle gel [15 16]

Based on the results of *in-vitro* release studies and entrapment efficiency formulation F3 selected as best formulation and further converted into gel and coded as GF3.

Evaluation of CS-NP incorporated gel (GF3) formulation

Physical examination

Visual appearance

The formulation GF3 was off-white colour and homogenous.

pH

The pH of the formulation GF3was found to be 7.2 and it is well in the range for ocular administration (Normal range 7.0-7.4).

Rheological studies

Viscosity of the CS-NP gel (GF3) was found to be 10945±80 cps. Rheological behaviour of the formulation GF3 indicated that the systems were non- Newtonian in nature showing decrease in viscosity at the increasing shear rates.

Percent drug content

The drug content of the formulation GF3 was found to be 95.20±0.34, which indicates the uniform of distribution of the drug throughout the formulation.

***In-vitro* release studies**

The *in-vitro* release studies of the formulation GF3 performed in phosphate buffer pH 7.4 and the results shows 82.15% over a period of 24hrs. The drug release study was compared with the marketed formulation. The marketed product showed complete release within 6hrs where as the formulation GF3 showed 82.15% release over 24hrs. The results are shown in the Figure 11.

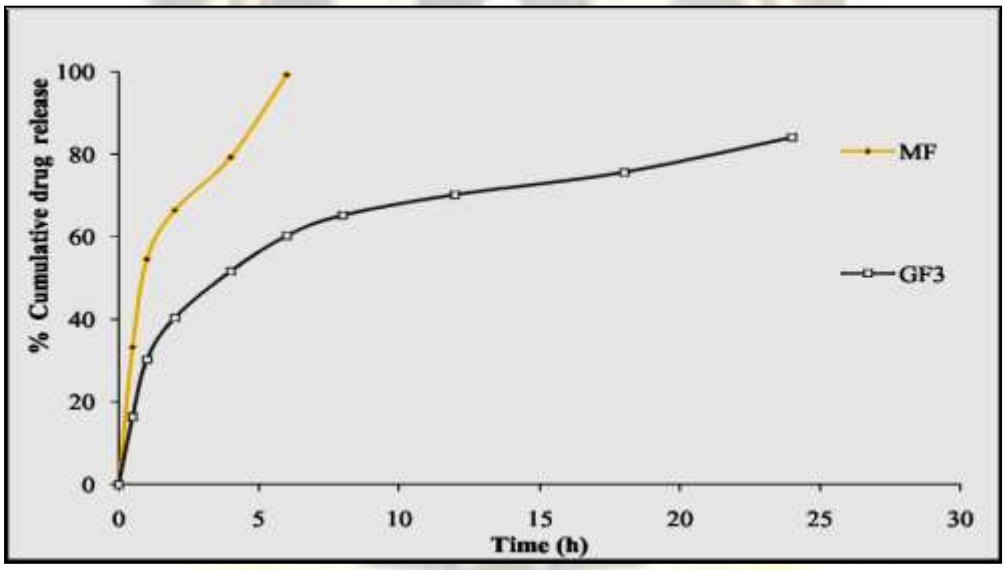

Figure 11: Comparative release studies of the CS-NP gel (GF3) with the marketed formulation

Kinetic model data analysis

The release data obtained from formulation GF3 was subjected for data analysis. The formulations followed Higuchi order release profile and the ‘n’ value was found to be more than 0.5. This indicates that the release approximates Non-Fickian diffusion mechanism. The results are shown in Table 2.

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

Optimized formulation Gel(GF3)	Zero order	First Order	Korsmeyer-Peppas	*'n'* value	Higuchi
Regression co-efficient(r^2)	0.7883	0.6027	0.9446	2.3417	0.9687

Antimicrobial efficacy studies[15, 16]

All the compounds were screened for their antibacterial activity by disc diffusion technique. Optimized formulation are screenedfor their *in vitro* anti-microbial activity against *Staphylococcus aureus* **(ATCC-9144)** and *Bacillus subtilis* **(ATCC -6051)** were compared with pure drug , standard drug amikacin (30µg) and marketed eye drops. From the results we have observed that the optimized formulation (30mm/20µg) showed higher zone of inhibition than the marketed product (28 mm/20µg) and pure drug (28mm/20µg) and the zone of inhibition is comparable to the standard drug which was used at 30µg). The results are shown in Figure 12, 13.

A) Pure drug **(B)Marketed product** **(C) formulation GF3**

Figure 12: Antimicrobial studies against *Staphylococcus aureus*

(A)Pure Drug **(B)Marketed product** **(C) formulation GF3**

Figure 13: Antimicrobial studies against *Bacillus subtilis*

Ocular irritation Studies[15,16]

Ocular irritation study was conducted for formulation GF3 as per Draize technique in male Albino rabbits. The results of the ocular studies indicated that the formulation GF3 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 Table 3-5.

Table.3: Eye irritation testing: Rabbit conjunctival observation

Redness	Normal rating	Rating for formulation(GF3)
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 4: Eye irritation testing: Rabbit iris observations

Values	Normal rating	Rating for formulation
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

Table 5: Eye irritation testing: Rabbit corneal observations for opacity and area of cornea involved

Opacity	Normal rating for	Rating for formulation GF3	Area of cornea involved	Normal rating for corneal area involved	Rating for formulation GF3
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

CONCLUSION

Emulsion solvent diffusion method is suitable to produce chitosan nanoparticle in nano metric size range. The drug Levofloxacin could very well be entrapped in the CS-NP and their characteristics could be monitored by making changes in various formulation and process variables. The Levofloxacin loaded chitosan nanoparticle gel showed an extended release when compare to marketed formulation. Antimicrobial studies revealed that the developed formulation have better antimicrobial action against *S. aureus* and *Bacillus subtilis* than the marketed formulation. Ocular irritation studies revealed that the developed formulation do not have produce any ocular irritation.

The developed formulation is hence suitable for sustained ocular drug delivery with better antibacterial action.

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REFERENCE

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