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Research Article

Formulation Development and Evaluation of Nanoparticulate Systems of Levofloxacin

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Abstract

An emerging advancement in pharmaceutical sciences and perturbing limitation of conventional drug delivery systems have triggered extensive research in novel carrier systems. There has been extensive research on novel carriers that promise patient compliance, but toxicological part is still the biggest challenge to any drug delivery systems. In the present research, we have develop levofloxacin nanopartulate systems with chitosan and sodium triphosphte which offer safer dosage form with increased bioavailability as well as better patient compliance. In all formulation the minimum percentage yield shows in formulation F8 (70.23%) and maximum in formulation F6 (89.23%). The highest % cumulative drug release after 8 hrs was found to be 92.658 and first order release kinetics; the r^2 value for first order was found to be 0.988

Keywords: Nanoparticulate systems, Chitosan, Levofloxacin, Compliance, Bioavailability.

INTRODUCTION

Nanoparticles have become one of the most active areas of research in the field of drug delivery due to their ability to deliver drugs to the right place, at appropriate times and in the right dosage.[1] They have received considerable attention over the past 20 years due to their advantages compared to other drug delivery systems. These advantages include: targeted delivery of drugs to the specific site to minimize toxicity; improved bioavailability by reducing fluctuations in therapeutic ranges; improved stability of drugs against enzymatic degradation; and the ease of administering through various routes including oral, nasal, pulmonary, intraocular, parenteral and transdermal.[2]

Bacterial conjunctivitis is inflammation of the conjunctiva caused by direct contact with infected secretions. The most common organisms are Staphylococcus species, S. pneumonia, H. influenzae, and M. catarrhalis.[11] It presents with conjunctival injection, mucopurulent discharge, and crusty eyelids. The diagnosis is usually clinical. The condition is often self-limiting, but there is good evidence that antibiotics improve remission rates.[12]

Ophthalmic formulation, levofloxacin is indicated for the treatment of bacterial conjunctivitis caused by susceptible organisms. Nanoparticle drug delivery strategies can play a essential role in improving the ocular delivery by enhancing their ophthalmic localization with a concomitant reduction in their side effects. Thus, the current strategy for enhancing the therapeutic activity of currently available drugs is to entrap drug within a delivery system from where they are slowly released over an extended time period.[3]

MATERIALS AND METHODS:

Levofloxacin was obtained as gift sample from FDC pvt. Ltd Aurangabad India. Different Polymers and excipients like Chitosan purchased from Thomas Baker Compony Pvt. Ltd. Whereas so Sodium Tripolyphosphate Loba Chemie Pvt.Ltd. All other ingredients used were of laboratory grade

Preparation of Chitosan nanoparticle [4]:

Chitosan nanoparticles were prepared according to the ionotropic gelation process Blank nanoparticles were obtained upon the addition of a tripolyphosphate (TPP) aqueous solution to a Chitosan solution (3mg/ml) stirred on 1000 rpm at room temperature. The formation of nanoparticles was a result of the interaction between the negative groups of TPP and the positively charged amino groups of chitosan. The ratio of chitosan/TPP was established according to the preliminary studies levofloxacin loaded nanoparticles were obtained according to the same procedure and the ratio of chitosan/TPP remained unchanged. Variable amounts of polymer were incorporated to the chitosan solution prior to the formation of nanoparticles in order to investigate the effect of the levofloxacin concentration on the nanoparticle characteristics and In- vitro release profiles. Nanoparticles were collected by centrifugation at 15,000 rpm

for a period of 30 minutes and supernatant was discarded. Followed by freeze drying, nanoparticles were collected

Formulation Development of Chitosan nanoparticle of Levofloxacin

Optimization of Chitosan nanoparticles

Table 1: Drug: Polymer ratio and Emulsifier concentration used

Batch No.	Drug: Polymer (w/w)	Emulsifierconc. (ml)
F1	1:2	0.75
F2	1:2	1.00
F3	1:2	1.25
F4	1:3	0.75
F5	1:3	1.00
F6	1:3	1.25
F7	1:4	0.75
F8	1:4	1.00
F9	1:4	1.25

Evaluation of Chitosan nanoparticle

Determination of Percentage Yield of Chitosan nanoparticle

Yield of nanoparticles, precent w/w was calculated according to the following formula:

% Yield = Weight of nanoparticles/ Wt. of drug + Wt.of excipients

Determination of entrapment efficiency [2]

The encapsulation efficiency and loading capacity of nanoparticles was determined by first separating the nanoparticles formed from the aqueous medium by ultra-centrifugation at 15000 rpm for 30 min. The amount of free Levofloxacin in the supernatant was measured by UV spectrophotometery at 368.0nm (Shimadzu UV 1700 + Spectrophotometer). The Levofloxacin entrapped in the nanoparticles was calculated as Eq 1.

$$M_t / M_\infty = kt^n$$

Entrapment efficiency (%) = (Tp – Tf) 100/Tp

Where Tp is the total Levofloxacin used to prepare the nanoparticles and Tf is the free Levofloxacin in the supernatant.

Determination of zeta potential [3]

The zeta potential of the drug-loaded chitosan nanoparticles was measured on a zetasizer (**Horiba Instruments**) by determining the electrophoretic mobility in a microelectrophoresis flow cell. All the samples were measured in water at 25 °C in triplicate.

Measurement of mean particle size [4]

The mean size of the nanoparticles was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer **(Horiba Instruments)** at a scattering angle of 90°. A sample (0.5mg) of the nanoparticle suspended in 5 ml of distilled water was used for the measurement.

Optical Microscope Observation [5]

The Nanoparticle dispersion was spread on the glass slide using a glass rod. Formation of nanoparticle was confirmed by examining under an optical microscope with the magnification power of 1000 x. Photographs of vesicles were taken using Olympus camera.

Determination of particle morphology

***Scanning electron microscope analysis* [5]**

Morphology and surface topology of the nanoparticles were examined by scanning electron microscopy. The nanoparticles from the optimized batch were mounted on glass slab with the help of adhesive tape. Sample was kept for scanning chamber.

Evaluation of *in vitro* drug release [7]

The Levofloxacin loaded chitosan nanoparticles, after separation by ultracentrifugation, were re- dispersed in phosphate buffer solution PBS solution (pH 7.4), placed in a dialysis membrane bag, tied and immersed in 50 mL of PBS and Methanol in the ratio of 4 :1 in a 100 ml beaker. The entire system was stirred continuously at 37 °C with a magnetic stirrer. At pre-determined time intervals, 3 mL of the release medium was removed and replaced with 3 mL of fresh PBS methanol solution. The amount of Levofloxacin in the release medium was evaluated by UV spectrophotometry at 368.0nm.

Release Kinetics:

A) Data analysis via drug release kinetics study

1. Cumulative amount of drug release versus square root of time (Higuchi model)
2. Log cumulative drug released versus log time (Korsmeyer-Peppas model)

1. Higuchi kinetics [7]

A plot of the fraction of drug released against root of time will the linear if the release obeys Higuchi Equation. This equation describes drug release as a diffusion process based on the Flick's Law, Square root time dependent

$Q=Kt^{1/2}$

Q=Amount of drug release per unit area in time t,K=release rate constant

2. Peppas & Korsemeyer equation [8]

The amount of drug released at time t (Mt) with respect to the total amount of drug released(M_∞), can be expressed in terms of an exponential expression as follows:

Where, M_t / M_∞ = The fraction of drug released at time t,

K = Constant incorporating the structural and geometrical characteristic of the drug /polymersystem.

n = diffusion exponent related to the release

RESULT AND DISCUSSION

1. Percentage Yield of Chitosan nanoparticles

Yield of nanoparticles percent w/w was calculated according to the following formula:

% Yield = Weight of nanoparticles/ Wt. of drug + Wt. of excipients

Table 2: Determination of Percentage Yield of Chitosan nanoparticles

Formulation Code	Percent (%) Yield
F1	77.23±0.23
F2	78.89±0.21
F3	77.56±0.56
F4	79.96±0.59
F5	80.23±0.87
F6	89.23±0.40
F7	72.26±0.56
F8	70.23±0.78
F9	71.23±0.25

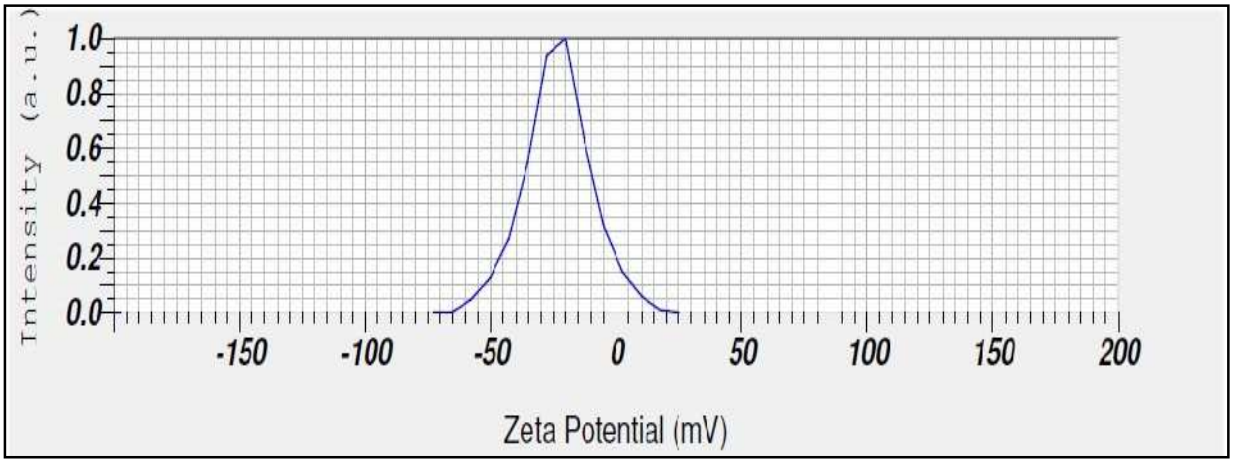
In all formulation the minimum percentage yield Found in formulation F8 (70.23%) and maximum in formulation F6 (89.23%). Hence on the basic of percentage yield formulation F6 was selected as an optimized formulation for further study.

2. a) Zeta Potential:

The zeta potential of the drug-loaded chitosan nanoparticles was measured on a zetasizer (Horiba Instruments) by determining the electro-phoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25 °C in triplicate.[10]

Results of zeta potential of optimized formulation F6 found: **-23.1 mV**

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-23.1 mV	-0.000179 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs



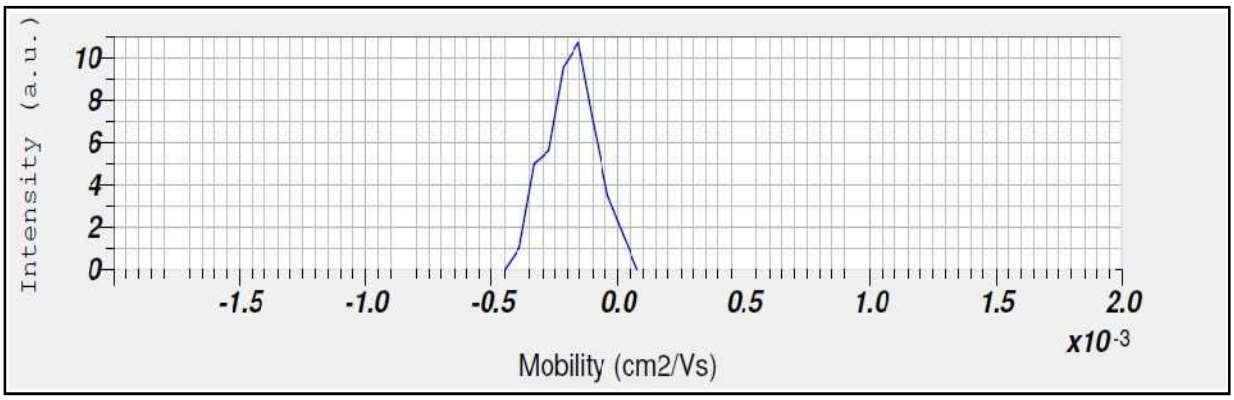


Figure 1: Zeta Potential

b) Mean Particle size*:*

The mean size of the nanoparticles was determined by photo correlation spectroscopy (PCS) on a submicron particle size analyzer (Horiba Instruments) at a scattering angle of 90°. A sample (0.5mg) of the nanoparticle suspended in 5 ml of distilled water was used for the measurement. The results of measurement of mean particle size found: **135.6 nm**

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	135.6 nm	32.3 nm	126.6 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	135.6 nm	32.3 nm	126.6 nm

3. Optical Microscope Observation:

The Nanoparticle dispersion was spread on a glass slide using a glass rod. Formation of nanoparticle was confirmed by examining under an optical microscope with the magnification power of 1000 x. Photographs of particles were taken using Olympus camera.

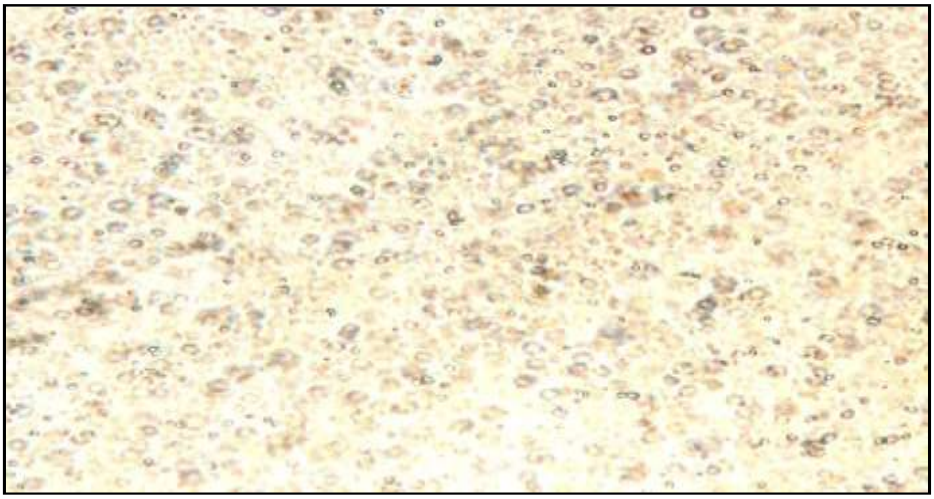


Figure 2: The microscopic image of Nanoparticles Scanning electron microscopic analysis

The scanning electron microscopic image of optimized batch (F6) was taken at SIF Lab Punjab University Chandigarh. A small amount of chitosan nanoparticle of Levofloxacin (Batch code F6) was mounted on glass slab with the help of adhesive tape. Sample was kept for scanning chamber. Photograph was taken as shown in Figure 3.

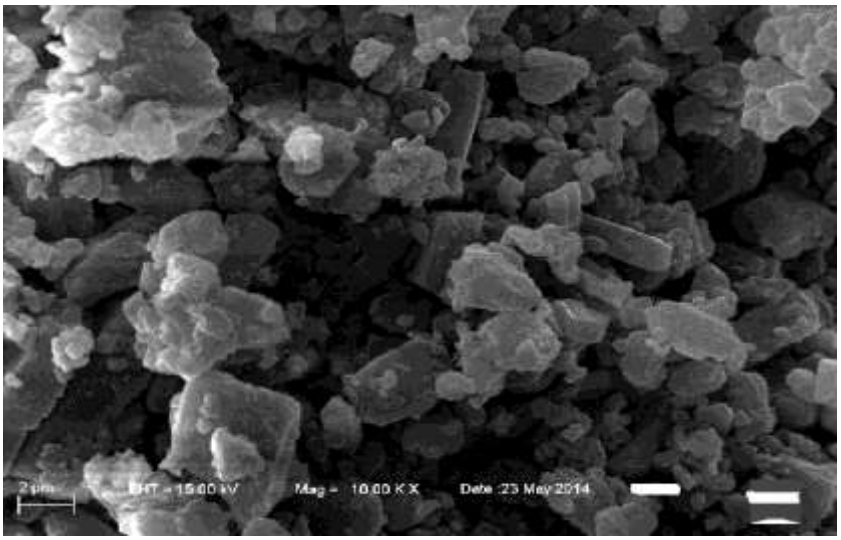


Figure 3: scanning electron microscopic image of nanoparticles of F6 formulation

4. Entrapment efficiency: [9]

Entrapment efficiency (%) = (Tp – Tf) 100/Tp

Where Tp is the total Levofloxacin used to prepare the nanoparticles and Tf is the free Levofloxacin in the supernatant.

The result of entrapment efficiency of all formulations were determined shown in table3 :-

5. In- vitro drug release:

Parameter: - Determination of release rate determine with the help of phosphate buffer (pH 7.4): Methanol (4:1). Buffer Volume were taken 50 ml and stirred at 75 rpm. Samples were taken out at different time interval eg. 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0 hrs and Replenishing volume taken out 3 ml.

Table 3: Determination of entrapment efficiency

S. No.	Formulation Code	% Entrapment efficiency
1.	F1	55.56 ± 0.13
2.	F2	56.89 ± 0.20
3.	F3	62.25 ± 0.23
4.	F4	65.25 ± 0.27
5.	F5	68.89 ± 0.56
6.	F6	86.56 ± 0.14
7.	F7	71.56 ± 0.26
8.	F8	70.12 ± 0.33
9.	F9	73.02 ± 0.69

***In-vitro* drug Drug release of optimized formulation F-6**

Table 4: Release study of formulation (F6)

S.No.	Time (in hrs.)	Absorption	%DrugRelease	CorrectionFactor	%CumulativeDrug Release
1	0.5	0.040	25.806	1.548	25.806
2	1	0.044	28.387	1.703	29.935
3	1.5	0.052	33.548	2.012	36.810
4	2	0.081	52.258	3.135	57.522
5	4	0.095	61.290	3.677	69.690
6	6	0.115	74.193	4.451	86.270

Higuchi release kinetics

Table 5: Higuchi release kinetics study of Formulation F6

S. No.	Square root of time (in hrs)	%Cum. Drug release
1	0.707	25.806
2	1	29.935
3	1.224	36.800
4	1.414	57.522
5	2	69.690
6	2.449	86.270

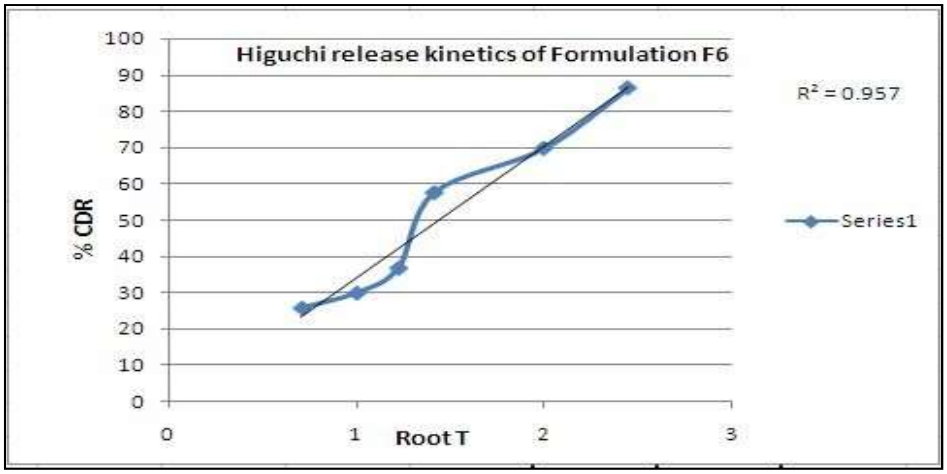


Figure 4: Graph of Higuchi release kinetics study of formulation F6

Peppas release kinetics data

Table 6: Peppas release kinetics study of Formulation F6

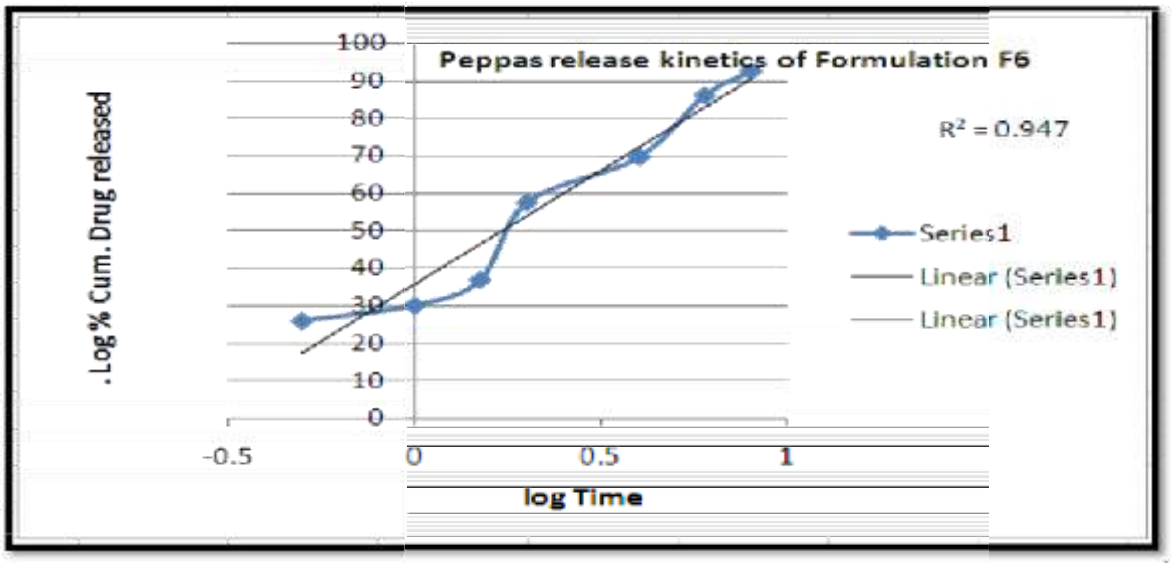


Figure 5: Graph of Peppas release kinetics study of formulation F6

Results of Comparative Parameters of release study:

Table 7: Correlation coefficient of Model fitting (R^2)

Formulationcode	Correlation coefficient of Model fitting (R^2)		Best fit model
	Higuchimatrix	Peppaskinetics	
F6	0.964	0.947	(First order) Model

CONCLUSION

The nanoparticulate systems of levofloxacin (F1-F-9) were developed successfully with the help of chitosan & sodium triphoshphate by ionotropic gelation process. The optimized formulation F-6 showing the cumulative percent drug release Vs square root of time (Higuchi plot) and log % cumulative drug release Vs log time (peppas release kinetic plot) were plotted separately. In each case, r^2 value was calculated from the graph and reported in table 7. The best fit model for formulation (F6) is first order release kinetics; the r^2 value for first order was found to be 0.988. It also has shown good stability in all storage condition.

CONFLICT OF INTEREST

Author declare that no conflict of interest

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