



# CIPROFLOXACIN HYDROCHLORIDE LOADED CHITOSAN NANOPARTICLE GEL: ADVANCED APPROACH FOR ENHANCING PERMEATION AND SUSTAINABILITY OF DRUG RELEASE

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## ABSTRACT

Transdermal drug delivery is one of the most reliable, appealing and effective technique which provides controlled and constant administration of drug. The aim of the study was to develop a gel form of nanoparticles loaded with ciprofloxacin hydrochloride in order to enhance the permeability of drug and for the release of drug over a period of 24 hrs. The nanoparticles were formulated by ionic gelation method using chitosan as a polymer and TPP as a cross linking agent. The compatibility of drug and polymer is studied by using FTIR spectroscopy and DSC method. There was no interaction observed by UV and FTIR study. The six different batches were prepared using different polymer and drug ratio. The fourth batch (N4) shows best results as compared to others which was used for further investigations. The formulation was then optimized for its particle size, zeta potential, morphology, drug content, drug entrapment efficiency, drug loading capacity and in-vitro permeation. TEM study reveals that the nanoparticles are spherical in shape and also confirms the size below 500nm. Drug release studies shows that nanoparticles could release drug for 24 hrs and follows zero order kinetics. From DSC analysis it was found that the drug was effectively encapsulated inside the chitosan nanoparticles. Finally, it was concluded that the penetration of ciprofloxacin hydrochloride was enhanced after loading it into chitosan nanoparticles and also the drug was release over 24 hrs.

**Keywords:** Nanoparticles, Ciprofloxacin Hydrochloride, Chitosan, Carbopol 934

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## INTRODUCTION

From last few decades, nanotechnology has appeared as a promising technique for various biomedical applications. Among all the nanomaterial's, nanoparticles have been investigated as a standalone agent as a novel carrier for delivery of therapeutic agents. Nanoparticles are the dosage form with size range of 1-1000 nm. Nanoparticles are an effective bridge between bulk material and atomic or molecular structures. The drug can be dissolved, entrapped, encapsulated or attached to the nanoparticle matrix. The major goals of designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacological agents in order

to achieve the site specific action of drug. Chitosan is a mucopolysaccharide obtained naturally by deacetylation of chitin, which is abundantly available in marine crustaceans. It is a biocompatible polymer which is used as a carrier due to its biocompatible and permeation enhancement properties.<sup>(1, 2)</sup>

## MATERIAL AND METHODS

Ciprofloxacin hydrochloride was a gift sample from Beta Max Pvt. Ltd, Tahlilwal and Chitosan was procured from LOBA Chemie Daryaganj, Delhi. Sodium tripolyphosphate was obtained from CDH Laboratory, New Delhi. Carbopol 934 was obtained from Hi Media Laboratories, Mumbai.

### Preformulation Studies:

Preformulation study is the process of optimizing the drug delivery through determination of various physicochemical properties of active compound which could affect the drug performance and development. It is an investigation to determine the physical and chemical properties of drug substance alone and with excipients to ensure its good quality. Preformulation study involves melting point determination, solubility and partition coefficient. The drug- excipient compatibility was carried using FT-IR spectroscopy.<sup>(3,4)</sup>

### Determination of $\lambda$ max

The absorption maxima of ciprofloxacin hydrochloride were scanned between 200-400 nm.  $\lambda$  max of the drug was determined by UV- visible spectrophotometric method to obtain the structural information regarding chromophoric part of ciprofloxacin hydrochloride.

### Fourier Transform Infra-Red (FTIR) spectroscopy

IR study was performed for identification and structural analysis of procured drug and polymer by using Perkin Elmer Fourier Transform infrared spectroscopy. The absorption maxima spectrum was compared with the reference spectrum.

### Differential Scanning Calorimetry (DSC)

The thermal behavior of drug, polymer, blank nanoparticles and drug loaded nanoparticles were studied using DSC. Any possible interactions can be determined by thermal analysis.

### Preparation of Standard stock solution of ciprofloxacin hydrochloride in saline phosphate buffer 7.4

Stock solution was prepared by dissolving 100 mg ciprofloxacin hydrochloride in phosphate buffer 7.4 and volume was adjusted to 100ml to produce a concentration of 1000 $\mu$ g/ml. From the prepared stock solution 10ml of solution was taken and volume was made up to 100ml for concentration of 100 $\mu$ g/ml. From the aliquots, a serial dilution was taken and made up to 10ml with phosphate buffer 7.4. Absorbance values of these solutions were measured against phosphate buffer 7.4 at 278 nm using UV- visible spectrophotometer.<sup>(5)</sup>

### Preparation of chitosan nanoparticles by ionic gelation method

Nanoparticles were prepared by ionic gelation method by combination of chitosan (0.2, 0.3 and 0.45) and TPP (0.3, 0.45 and 0.65). Six formulations were prepared using different drug and polymer ratios of 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6. Chitosan was dissolved in acetate buffer 4.5 and TPP was dissolved in phosphate buffer 7.4. Chitosan was added dropwise to an equal volume of sodium tripolyphosphate solution under magnetic stirring for 60 minutes. Drug loaded nanoparticles were prepared by same method, but the appropriate amount of ciprofloxacin hydrochloride was dissolved in chitosan solution before the addition of TPP.<sup>(6-9)</sup>

### Characterization of ciprofloxacin hydrochloride loaded chitosan nanoparticles

#### Particle size and zeta potential

Particle size, zeta potential and polydispersity index of nanoparticle formulation were measured using Zetasizer

Nano- Series Nano- ZS (Malvern Instruments, UK). Each sample was measured in triplicate and average results were calculated.<sup>(10, 11, 12)</sup>

### Drug entrapment efficiency

Entrapment efficiency was calculated by measuring the amount of free drug left in supernatant after centrifugation based on absorbance of the sample at 278 nm. The standard curve was obtained using Shimadzu UV- 1800 UV- Visible spectrophotometer.<sup>(13)</sup>

### Drug content

To determine the drug content, about 1 mg of pure drug was dissolved in ethanol and volume was made upto 100ml with ethanol and diluted appropriately. The drug content was determined with UV spectrophotometer.

### Drug loading capacity

The drug loading capacity was determined from ciprofloxacin hydrochloride in nanoparticle sediment.<sup>(14)</sup>

$$\%DL = \frac{\text{Amount of drugs in nanoparticles}}{\text{Amount of drug added (mg) + Amount of excipient added}}$$

### In- vitro drug permeation study

In vitro drug permeation studies were performed by using Franz Diffusion cell of a period of 12 hrs. The goat skin was placed on the diffusion and the receiver compartment was filled with saline phosphate buffer 7.4 and 37°C temperature was maintained. At appropriate time intervals the samples were withdrawn with the help of syringe. The samples containing released drug were withdrawn from acceptor compartment and measured at 278 nm by using UV- Visible spectroscopy.<sup>(15)</sup>

### Fourier- Transform Infrared Spectroscopy(FTIR)

FT-IR spectroscopy was performed to determine various types of bonds present in nanoparticles. FT-IR spectra of chitosan nanoparticles and chitosan- ciprofloxacin hydrochloride nanoparticles were performed using KBr pellets on a Perkin Elmer 1600, USA.<sup>(16)</sup>

### Differential Scanning Calorimetry (DSC)

DSC thermogram of pure drug and polymer mixture were performed to check any possible interaction between drug and other additive.

### Transmission Electron Microscopy (TEM)

TEM studies were performed to determine the size, shape and morphology of the unloaded and loaded chitosan nanoparticles.

### Evaluation of nanoparticles loaded gel

The prepared nanoparticles loaded in gel were characterized for their pH, spreadability, gel strength, viscosity and in vitro permeation.<sup>(17, 18)</sup>

## RESULT AND DISCUSSION

### Preformulation Studies

Ciprofloxacin hydrochloride was found to be white powdery substance.

### Solubility

The solubility of drug was checked, it was found that the drug was highly soluble in water (1.13mg/ ml), soluble in ethanol (0.3 mg/ml) and poorly soluble in acetone.

### Melting point

Melting point of drug was measured in triplicates and the mean was found to be 313<sup>0</sup>C which was same as pharmacopoeial reference.

### Determination of absorption maxima

Absorption maxima of ciprofloxacin hydrochloride in phosphate buffer 7.4 were found to be 278nm which resembled with pharmacopoeial standards.

### Fourier Transform Infra-Red Spectroscopy

The FT-IR spectrum of ciprofloxacin HCl, chitosan and mixture of ciprofloxacin HCl and chitosan was showed in Figure 1, Figure 2 and Figure 3

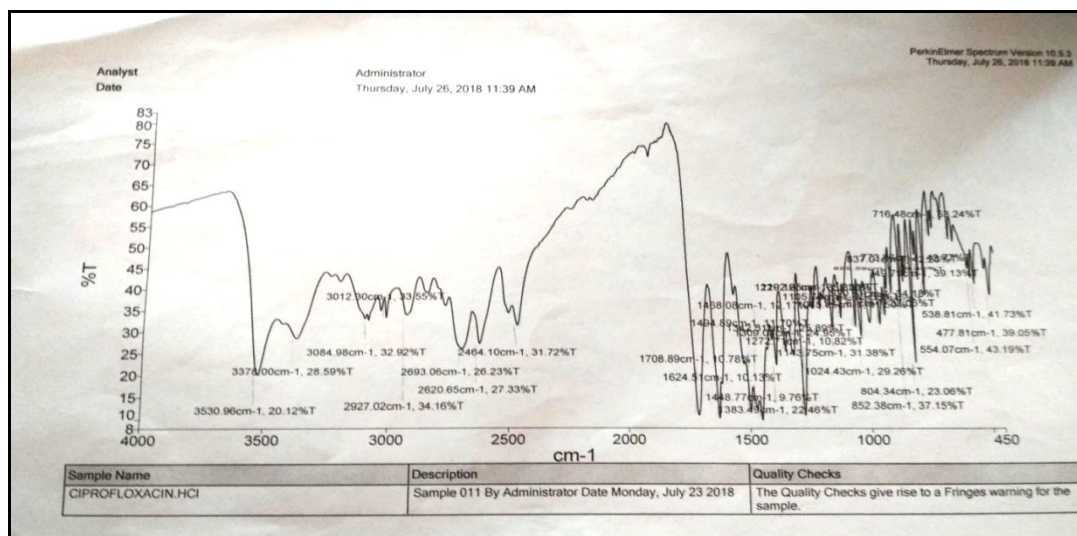


Figure 1: FTIR spectrum of Ciprofloxacin Hydrochloride

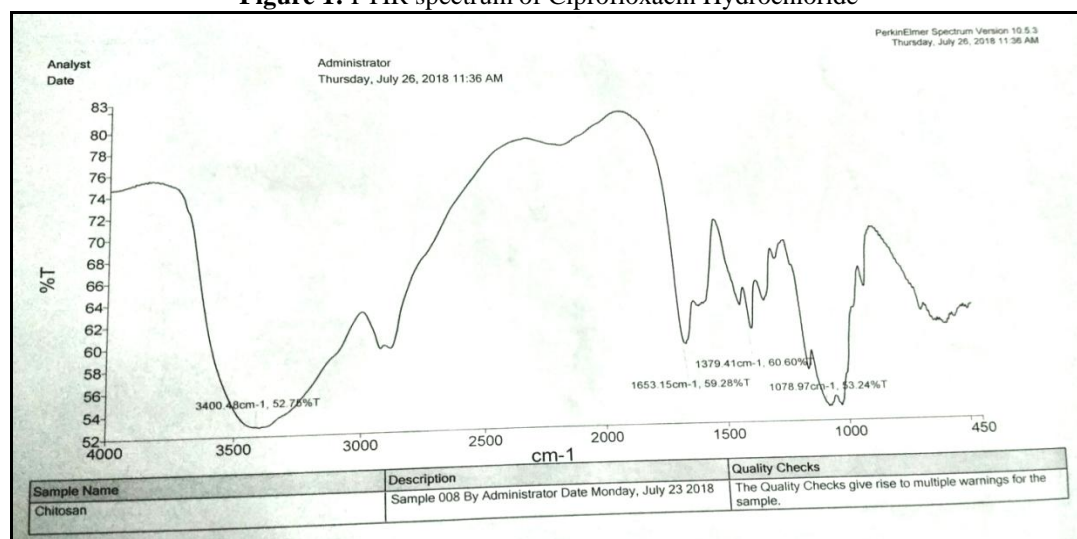


Figure 2: FTIR spectrum of Chitosan

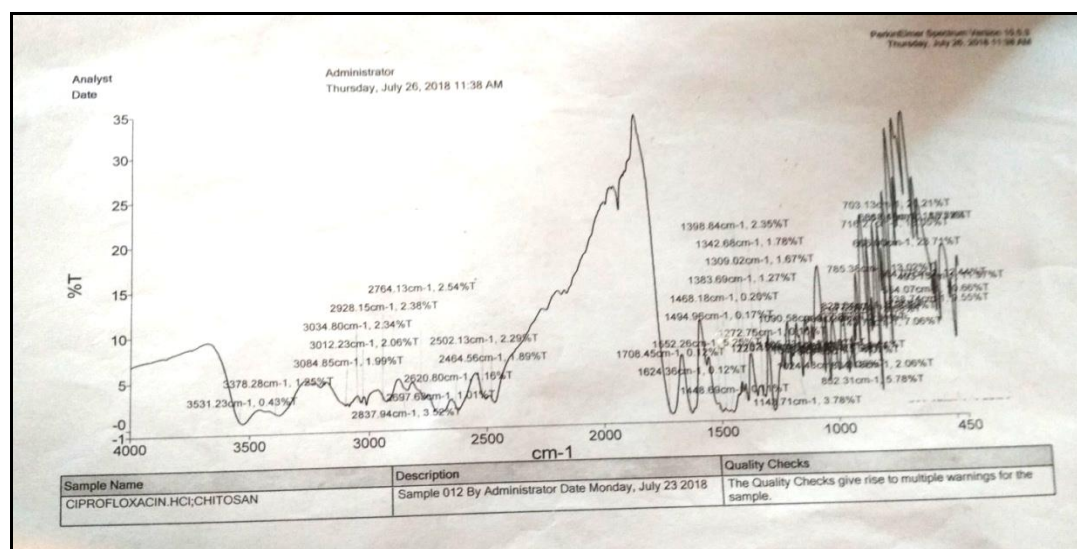
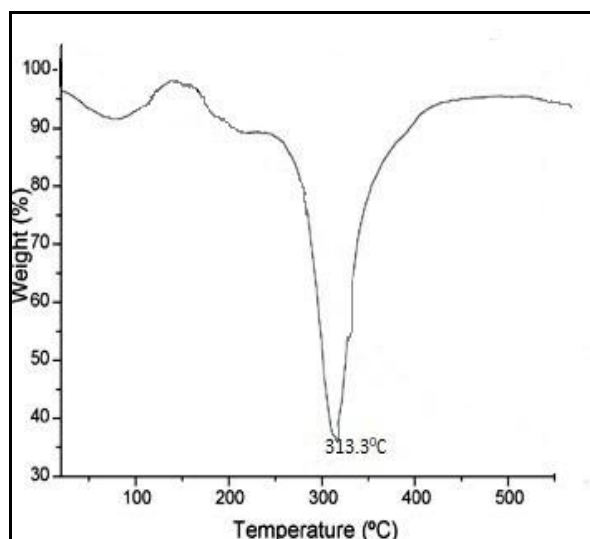


Figure 3: FTIR spectrum of Ciprofloxacin HCL + Chitosan

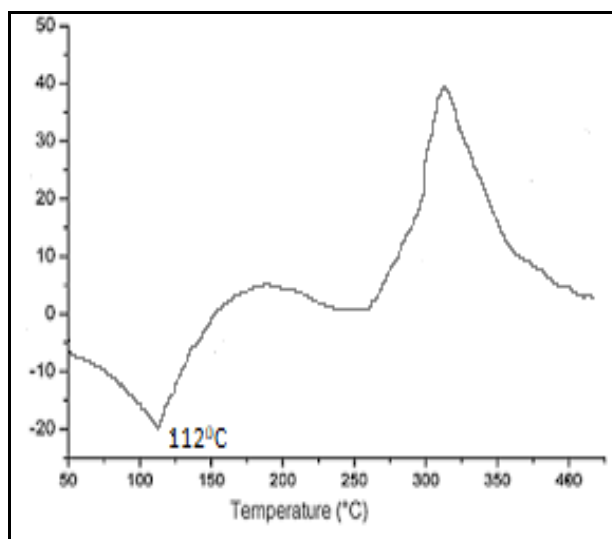


### Differential Scanning Calorimetry (DSC) studies

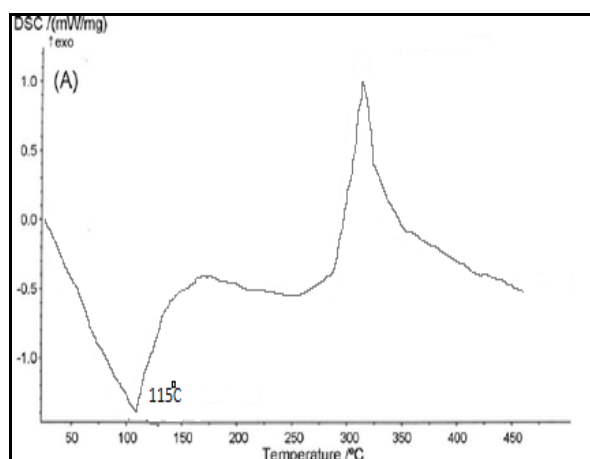
Thermal behavior of pure drug, polymer, unloaded nanoparticles and loaded nanoparticles are showed in **Figures 4**, **Figure 5**, **Figure 6** and **Figure 7**.



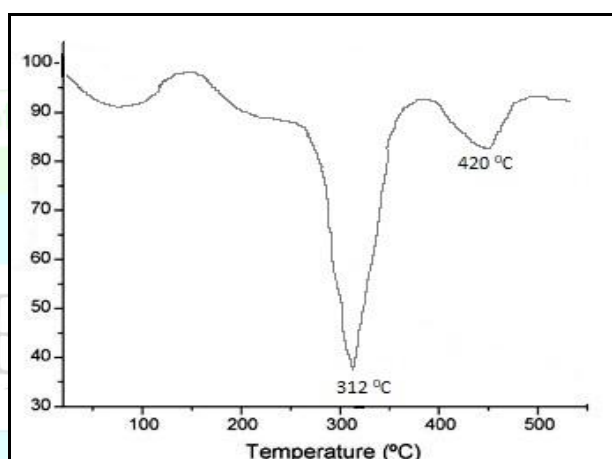
**Fig. 4:** DSC thermogram of Ciprofloxacin Hydrochloride



**Fig. 5:** DSC thermogram of Chitosan



**Fig. 6:** DSC Thermogram of unloaded chitosan nanoparticles



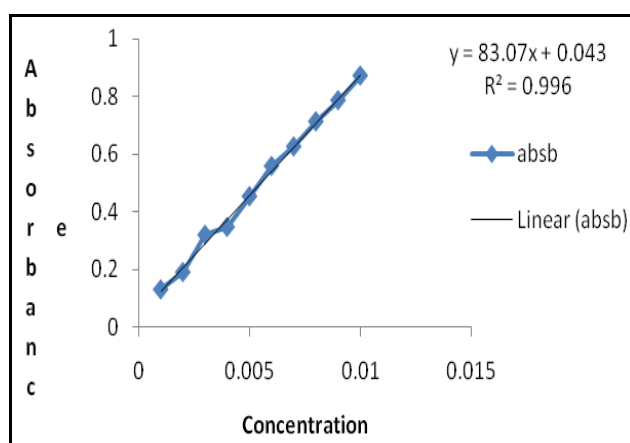
**Fig 7:** DSC Thermogram of drug loaded nanoparticles

### Standard curve of ciprofloxacin hydrochloride in saline phosphate buffer 7.4

A straight line was obtained by plotting the concentration of ciprofloxacin HCl (µg/ml) versus absorbance. **Figure 8** showed standard curve of ciprofloxacin hydrochloride.

### Formulation and Optimization of Ciprofloxacin Loaded Chitosan Nanoparticles

Nanoparticles were prepared by ionic gelation method. Formulations were optimized on the basis of particle size, zeta potential, polydispersity index, drug content, entrapment efficiency and drug loading capacity.



**Fig. 8:** Standard curve of Ciprofloxacin Hydrochloride in Phosphate Buffer 7.4**Table 1:** Optimization of Chitosan: TPP ratio

S No.	Factors			Experimental Result		
	CS Conc. (%)	TPP Conc. (%)	Stirring Speed (rpm)	Particle size (nm) mean±SD	Average PDI (mean±SD)	Average ZP (nm) (mean±SD)
1	0.2	0.3	6000	400.77±117.33	0.28±1.290	20.82±8.9
2	0.2	0.45	9000	322.64±112	0.56±1.110	22.79±6.8
3	0.2	0.65	13500	451.13±160	0.25±1.49	18.06±9.8
4	0.3	0.3	9000	215.00±94	0.16±0.78	43.88±4.8
5	0.3	0.45	13500	192.66±623.3	0.27±0.710	12.02±18.7
6	0.3	0.65	6000	252.89±111.6	0.58±1.103	78.69±15.3
7	0.45	0.3	13500	219.51±338	0.59±1.17	70.64±6.5
8	0.45	0.45	6000	213.32±71.6	0.57±1.05	65.62±2.4
9	0.45	0.65	9000	283.74±15.4	0.67±0.001	62.02±6.5

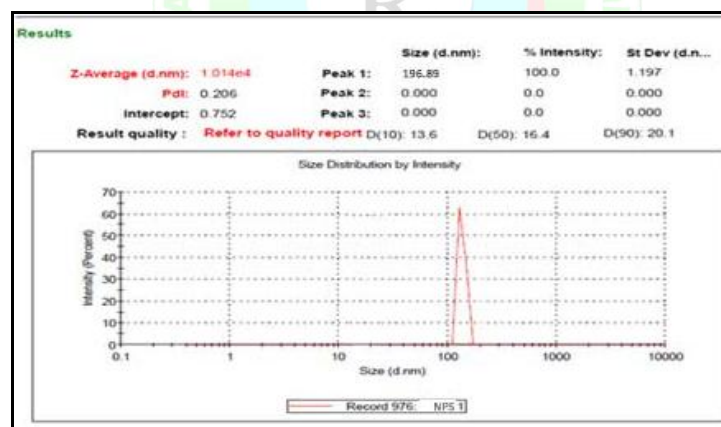
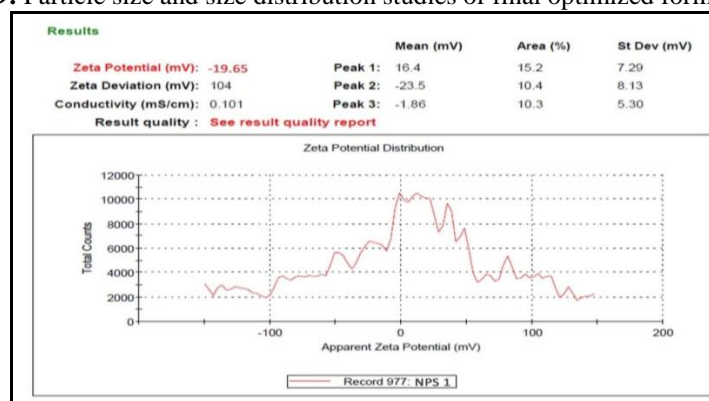
The particles prepared under prepared under conditions of 0.3% chitosan concentration and 0.65% of TPP under stirring speed of 6000 rpm shows best results with 252.89 particle size, 0.58 PDI and 78.69 mv of zeta potential.

**Table 2:** Optimization of ciprofloxacin hydrochloride loaded chitosan nanoparticles

S NO.	Chitosan: Ciprofloxacin HCL Ratio	Particle Size (Nm) ±Sd	Average Pdi ± Sd	Average Zp (Mv)	Amount (Mg)	Ee (%)	Drug Loading (%)
	Drug free particles	200.40±20.8	0.20±19.23	-28.22± 2.36	0	0	0
N1	1:1	299.56±70.6	0.25±0.02	-50.48± 5.06	27.96±0.36	64.69	79.01
N2	1:1.5	257.98±20.1	0.22±0.06	-55.36± 3.01	57.41±0.28	71.53	85.23
N3	1:2	710.3±26.30	0.78±0.07	-14.86± 5.04	10.67±0.08	75.24	81.56
N4	1:2.5	194.60±31.2	0.206±0.05	-19.65± 2.66	14.96±0.087	80.59	71.62
N5	1:3	199.62±5.44	0.68±0.05	-40.88± 2.26	15.23±0.21	85.86	70.53

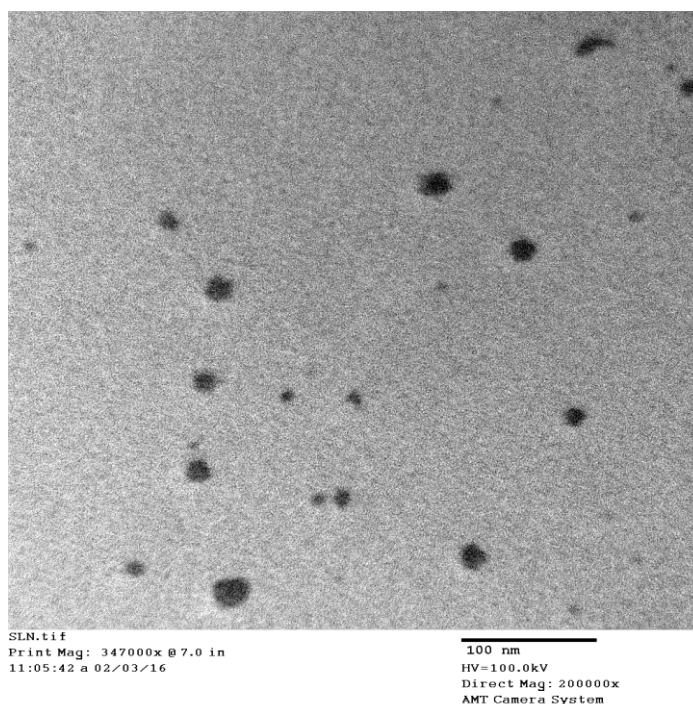
There was increase in zeta potential showed after addition of drug (ciprofloxacin hydrochloride). The **Particle size and Size distribution analysis**

ratio of 1: 2.5 showed least particle size and was used for further experiment.

**Fig. 9:** Particle size and size distribution studies of final optimized formulation**Fig. 10:** Zeta potential of final optimized formulation

### Morphology of Ciprofloxacin Hydrochloride loaded Nanoparticles

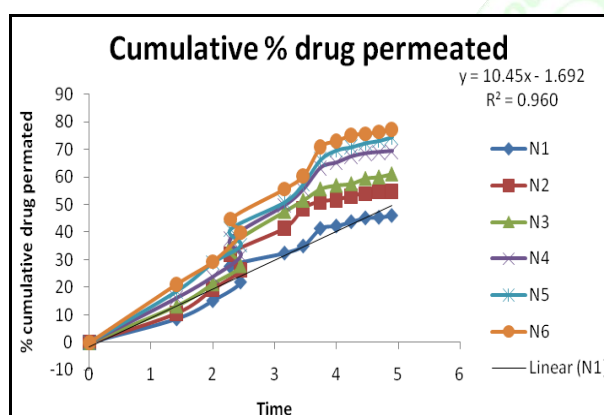
Transmission Electron Microscopy (TEM) was used to study the morphology of ciprofloxacin hydrochloride loaded nanoparticles.



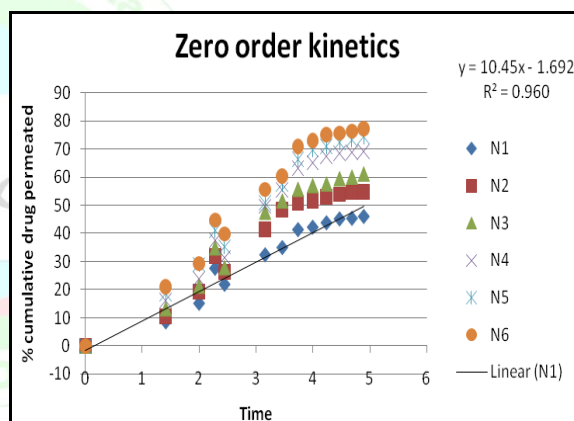
**Fig. 11:** TEM of final optimized formulation

### In vitro permeation studies

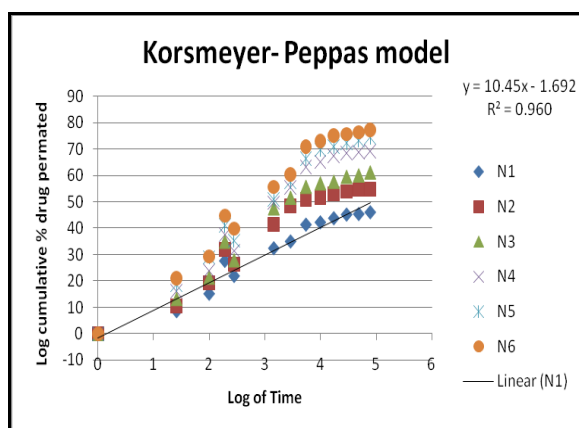
The in vitro permeation of ciprofloxacin hydrochloride from nanoparticles by goat skin in saline phosphate buffer pH 7.4 was observed.



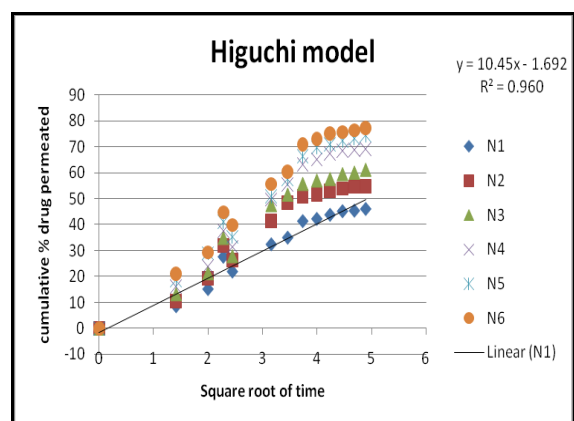
**Fig. 12:** In Vitro Drug Release Profile of Different Chitosan Nanoparticle Formulations



**Fig. 14:** Drug Release Kinetics of Optimized Formulations (First Order Kinetics)



**Fig. 15:** Drug Release Kinetics of Optimized Formulations (Korsmeyer – Peppas Model)



**Fig. 16:** Drug Release Kinetics of Optimized Formulations (Higuchi Model)

### Preparation And Physical Evaluation Of Gel Containing Nanoparticles

Carbopol 934 was used as gelling agent for preparation of gel. The gel was prepared in three different

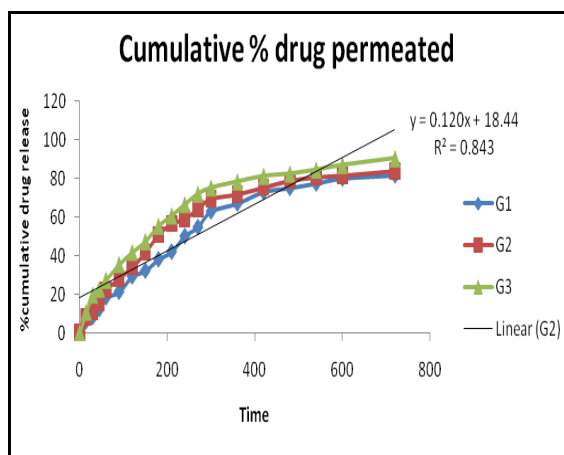
concentrations (1.0%, 1.5% and 2.0%) and added to optimized nanoparticle formulation under continuous stirring.

**Table 3:** Physical Evaluation of Gel Containing Nanoparticles

Formulation Code	Ph	Spreadability	Gel Strength	Viscosity	Permeability Coefficient
G1	6.41±0.040	1.54±0.086	60±3.12	29.13±0.48	6.1
G2	6.73±0.086	2.94±0.070	89±3.09	35.51±0.80	6.3
G3	6.90±0.094	6.77±0.196	130±3.00	38.65±0.75	6.7

### In vitro permeation study of gel containing nanoparticles

In vitro permeation study of drug from gel was carried out using goat skin by Franz diffusion cell in saline phosphate buffer 7.4.



**Figure 17:** In vitro drug release profile of drug loaded nanoparticles gel

### Stability studies of ciprofloxacin hydrochloride loaded chitosan nanoparticles

The stability of prepared nanoparticles was tested on storing them in glass vials at 3-8°C and 25°C for 28 days. They were evaluated for particle size, zeta potential and entrapment. After storage for 30 days, it was concluded that the formulation stored at 2-8°C was more stable as compared to those stored at 25°C. The entrapment efficiency also reduced at 25°C. So the storage temperature for stable formulation was found to be 2-8°C.

### CONCLUSION

The ciprofloxacin hydrochloride loaded chitosan nanoparticles were formulated with six ratios of drug and polymer mixture. Identification and interaction of drug, polymer and drug polymer mixture was studied with FTIR and DSC which showed no interaction which meant that drug is compatible with polymer. The six batches were evaluated for their particle size, zeta potential and entrapment efficiency. The fourth batch (N4) with ratio 1:2.5 of drug and polymer mixture was found to be best which showed least particle size, highest encapsulation and follows zero order kinetics. The TEM study showed spherical nature and size of drug loaded nanoparticles. The release kinetics revealed that the prepared formulation follows zero order kinetics and due to bioadhesive nature of chitosan there was depot formation

which could release drug in prolonged fashion of 24 hrs. So, it was concluded that ciprofloxacin HCl loaded chitosan nanoparticle gel improved the permeation and prolonged the release of drug over period of 24 hrs.

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