CIPROFLOXACIN HYDROCHLORIDE LOADED CHITOSAN NANOPARTICLE GEL FOR TREATMENT OF ACNE VULGARIS: NOVEL APPROACH FOR ENHANCING PERMEATION AND SUSTAINABILITY OF DRUG RELEASE

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Interest of Study: The scope of present research study is to develop a topical drug delivery for the drugs which have low solubility and also have permeability issues, Ciprofloxacin hydrochloride has been selected for purpose of enhancing permeability and sustainability of drug. The antibacterial drug was loaded with chitosan nanoparticles. Ion-Gelation method is use for formulation of nanoparticles using chitosan as a polymer and TPP as crosslinking agent.

Significance of research work: The permeability of Ciprofloxacin Hydrochloride can be enhanced when incorporated with chitosan nanoparticles. The solubility of drug can be improved when we use salt form of it. The prepared chitosan loaded nanoparticles incorporated in topical dosage form which will bypass the hepatic first pass metabolism. Development of ciprofloxacin loaded chitosan nanoparticles shows the synergistic antibacterial activity and eventually it avoids the adverse effect by minimising the dose.

Abstract:

Nanotechnology is an emerging interdisciplinary revolution in several therapeutic areas over the last decade, including medicine, and the drug delivery system. The essence of this new technology features a significant impact within the field of diagnosis and drug delivery. We developed chitosan nanoparticles to entrap ciprofloxacin hydrochloride and evaluate for the first time the impact of this polymeric nano system on the targeted drug delivery to the pilosebaceous units, considering the sebaceous characteristics of skin affected by acne. One of the most appealing, dependable, and efficient methods for consistently providing regulated drug administration is transdermal drug delivery system. The current study's goal was to create ciprofloxacin hydrochloride-loaded gel nanoparticles in order to increase their permeability and facilitate drug release over a 12-hour timeframe. Ion-Gelation method is use for formulation of nanoparticles using chitosan as a polymer and TPP as crosslinking agent. The compatibility of drug and polymer is studied 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. Impact of different formulation factors on crucial qualities including entrapment efficiency were studied using a full factorial design. Optimized formulation shown (+19.32) mV zeta potential and a mean particle size (189.8) nm. When compared to Ciprofloxacin Hydrochloride solution, its nanoparticles exhibited a considerable increase in transdermal penetration in ex-vivo diffusion study across isolated rat skin.

Keywords: Acne vulgaris; polymeric nanoparticles; skin penetration; Ciprofloxacin Hydrochloride; Carbopol 940.

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Introduction:

One of the most prevalent skin conditions is acne.[1] Significant deformity in young individuals can have catastrophic consequences, including despair and suicide. Acne vulgaris is the second most typical skin condition to cause suicide. Acne sufferers are found to be more anxious, more aggressive, and have higher levels of socio-inhibition than those without the condition.[2] The skin condition known as acne develops when the sebaceous glands (SGs) become overactive. Neither men nor women are afflicted by this illness; nevertheless, the symptoms are more severe in men.[3]According to data from 2010, 9.4% of the population suffers from acne.[7] Over 90% of people have it during adolescence, and sporadically into adulthood.[4] Approximately 20% of the population is affected by moderate to severe cases. Acne is uncommon in rural areas, and non-Westernized Paraguayans and Papua New Guineans may not have it.[6] In comparison to men's 9.0% prevalence, women's 9.8% prevalence is higher.[7] In subjects over 40, about 1% of men and 5% of women report having problems.[5] While it is unknown whether race has an effect on disease rates, it affects people of all racial and ethnic backgrounds.[8,9] In the United States, 40 to 50 million people, or around 16 percent of the population, suffer from acne. In Australia, 3 to 5 million people, or roughly 23 percent of the population, suffer from acne.[10]

The many over-the-counter and prescription treatments for acne allow for a large number of potential combination treatments. According to a thorough systematic review, topical therapy is typically used as the first line of treatment for moderate acne, while systemic therapy is crucial in addition to topical therapy for severe and complicated acne. There are various formulas for managing acne, but the most of them don't have treatments that have been clinically validated. Successful treatments take around three months to improve and start flattening out, with minimal progress seen in the first two weeks.[11]

At least four main methods of treatment are thought to be effective in managing and controlling acne: enhances shedding into the pore to maintain normal function, destroys P. acnes, Responses that are anti-inflammatory, hormonal regulation.

Materials and method

Materials

Ciprofloxacin Hydrochloride was received as gratis sample from Zim Laboratories Limited, India. Chitosan and Sodium tripolyphosphate was purchased from Merck, India. Carbopol 940 Extra pure grade was purchased from Loba Chemie Private Limited, India. HPLC grade chloroform and methanol were purchased from Merck, India. All other reagents and chemicals used were of analytical grades.

Experimental method:

Method

Experimental design

Optimization of formulated batches by using 3^2 Design

Full factorial design for two factors at three levels each was selected to optimize the response of variables. The two factors, the precursor Chitosan (mg) and TPP (Sodium tri polyphosphate respectively) used varied and the factor levels were suitably coded. The particle size, poly dispersibility index and Zeta potential were taken as the response variables. In the design, the two factors were evaluated each at three levels and experimental trials were executed for possible combinations. All other formulation variables and processing variables were kept constant throughout the studies (Aburjai et al., 2019). All the formulated batches were optimized by taking 3 responses into consideration. The sets of the variables following 32 full factorial design (3 levels) using Design expert. Dependent variables were Y1=Particle size, Y2=PDI, Y3 =Zeta Potential.

The influence of independent variables: concentration of chitosan(X1) and concentration of TPP(X2) on the dependent variable: entrapment efficiency was investigated using a 3^2 full factorial design with 9 different combinations of trials. Table 1 lists formulation variables based on the experimental design.

Preparation of chitosan nanoparticles

Chitosan nanoparticles were prepared by ionic gelation method by combination of chitosan (0.25mg/mL, 1.125mg/mL and 2mg/mL) and TPP (0.25mg/mL, 0.625mg/mL and 0.65mg/mL). Nine formulations were prepared using different drug and polymer ratios. Chitosan was dissolved in the solution of 1% acetic acid and kept overnight on magnetic stirrer for 12 hours, drug was added to the solution of chitosan and pH was adjusted to 4.7 by using 0.1N NaOH solution. TPP solution was added dropwise to above solution under magnetic stirring for 60 minutes.[12]

Particle size and Zeta potential measurements

Photon correlation spectroscopy with dynamic light scattering on a Zetasizer ® Nano (Model:

ZS, Malvern Instrument, Malvern, UK) with a wavelength output of 633 nm and Malvern PCS software was used to determine mean particle diameter, polydispersity index and zeta potential of BCM entrapped UDL. The investigation was conducted at 25°C at a 90° angle with a total run time of 500 seconds using deionized water as dispersant.[13]

Entrapment efficiency (EE)

Ultracentrifugation technique was used to assess entrapment efficiency of Ciprofloxacin Hydrochloride loaded nanoparticles. Samples were centrifuged at 15000 rpm for 30 minutes using colling centrifuge (Remi C-24, Mumbai, India). Unentrapped Ciprofloxacin Hydrochloride content in supernatant was diluted with an appropriate medium before being measured using a UV-Visible spectrophotometer. Entrapment efficiency was calculated as per equation 1.[14]

$$\text{Entrapment Efficiency} = \frac{\text{Free Drug} - \text{Total Drug}}{\text{Total Drug}} \times 100$$

Field Emission Scanning Electron Microscopy study

Surface morphology of Ciprofloxacin Hydrochloride entrapped nanoparticles was studied using field emission scanning electron microscopy (FE-SEM). Ciprofloxacin Hydrochloride entrapped nanoparticles were placed on silica chip and images were taken using FE-SEM (Jeol JSM- 7610F, Japan) at an acceleration voltage of 5Kv.[15]

Transmission Electron Microscopy

TEM studies were performed to determine the size, shape and morphology of the Ciprofloxacin HCl entrapped chitosan nanoparticles (JEOL JEM-1400, Japan).

FT-IR spectroscopy study

FT-IR spectrum of Ciprofloxacin Hydrochloride, Chitosan and optimized Ciprofloxacin entrapped nanoparticles were recorded using FT-IR spectrophotometer (Jaco FT-IR 6700) using KBr press technique. About 1-3 mg of sample was mixed with dry potassium bromide in 1:1 ratio and samples were scanned at transmission mode over wave number range 4000-400 cm^{-1}.

Differential scanning colorimetry study

Differential scanning colorimetry studies for Ciprofloxacin Hydrochloride, Chitosan and optimized formulation were conducted individually. Study was carried out using DSC instrument (Universal V4.5A TA Instruments, Diya Lab, Mumbai, India) and (STARe Thermal Analysis Software Ver.10, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India). Samples (5mg) were placed in aluminium crucibles (Al-Crucibles, 40 Al) and sealed. The probes were heated from 25 to 400°C at heating rate 10°C/min in an inert nitrogen atmosphere.[16]

Determination of minimum inhibitory concentration (MIC) of Ciprofloxacin Hydrochloride: [27]

The minimal inhibitory concentration (MIC) value is the lowest concentration of the compound to inhibit the growth of microorganisms.

Preparation of Nutrient Agar media

37 gm of Nutrient Agar (NA) was dissolved in 1000ml of distilled water as given in the manufacturer's instructions and heated till boiling. Then it is autoclaved for 20 minutes at temperature 120°C and 15 psi. After autoclaving, the agar media was cooled to 40-45 °C in a water bath. Then the cooled agar media was poured onto petri dish and then allowed to cool down until the media gets solidified.

Preparation of Dilution Sample:

Preparation of Inoculum:

The sub cultured slants of *S. aureus* were obtained from Rajiv Gandhi Biotechnology Park, Nagpur. One or two isolated colonies of the tested organisms were touched using sterile cotton swab. Bacterial strains were suspended in a nutrient broth media and then the suspension adjusted to turbidity to 0.5 McFarland standard either by adding more microorganism if the suspension was too light or diluted with sterile saline if the suspension was too dark. The suspension was prepared before inoculating the microorganisms on the plate.

Inoculation of Nutrient Agar plate:

To inoculate NA agar plates, a sterile cotton swab was dipped into the suspension and streaked over the surface of the agar plates. The plate with swab was rotated such that the inoculum was evenly distributed. The plates were allowed to settle at room temperature for 5 min before applying the extract.

Preparation of Agar Well Diffusion Assay:

The antibacterial activity was determined using agar well diffusion assay. A sterile cork borer was used to make wells of equidistance in each of plates were cut into which 100 µl of solutions of different

concentrations were introduced in each well. The inoculated agar plate was incubated at 37 ºC for 24 hours. The antibacterial activity was evaluated by measuring the diameters of zone of inhibition (in mm) by using ruler.

***Ex-vivo* diffusion study across isolated skin**

Preparation of whole skin:

Before approval was obtained from Institutional Animal Ethics Committee, University Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur (Protocol N. IACE/UDPS/2021/18) for preparation of whole skin. Excess ether inhalation was used to sacrifice healthy adult male Wistar rats with an average body weight 240-300g. Animal hair clippers were used to remove hairs on dorsal side skin. Skin of animal was removed and adhered tissues were separated using a scalpel blade. Whole skin was cleaned with normal saline, covered in aluminium foil and kept in freezer at (-) 20 °C until use.[17]

Diffusion study:

Using a 6-cell Franz diffusion assembly (Orchid Scientific, India), drug diffusion through rat skin was investigated. Briefly, prepared whole rat skin was kept in a thermostatic bath shaker for 1 h at 37±1 °C and placed between the donor and receptor compartments of diffusion cell on which developed Ciprofloxacin Hydrochloride entrapped nanoparticles was added. To the receptor compartment 20mL phosphate buffer pH 7.4 was added, maintained at 37±0.5 °C and was subjected to constant stirring at 100 rpm. At time intervals 1,2,4,6,8,10,12 h samples were withdrawn and replaced with an equivalent volume of phosphate buffer pH 7.4. Aliquots were spectrophotometrically analyzed for the amount of drug diffused through skin at 277nm using UV 1800 spectrophotometer (Shimadzu, Japan).[18]

In-vitro drug release study

Using a 6-cell Franz diffusion assembly (Orchid Scientific, India) with a receptor compartment capacity of 20 mL and dialysis membrane-110 (pore size 0.45), an in-vitro drug release study was conducted. Dialysis membrane was mounted between the donor and receptor compartments of the diffusion cell and on that prepared Ciprofloxacin Hydrochloride entrapped nanoparticles suspension equivalent to 10 mg Ciprofloxacin Hydrochloride was loaded. Donor compartment was placed on receptor compartment and wrapped with aluminium foil to prevent contamination. Receptor compartment was filled with 20 mL phosphate buffer pH 7.4 and entire assembly was mounted on a hot plate magnetic stirrer set to 100 rpm to continually stir the solution in the receptor compartment. At time interval 1, 2, 4, 6, 8, 10, 12h samples (1 mL) were withdrawn and replaced with an equal volume of phosphate buffer pH 7.4. Aliquots were spectrophotometrically analyzed for amount of drug released at 277 nm using UV-1800 spectrophotometer (Shimadzu, Japan).[19]

Formulation of Ciprofloxacin Hydrochloride entrapped nanoparticles loaded gel

To extend the epidermal retention duration, an optimized Ciprofloxacin HCl entrapped nanoparticle suspension was chosen for conversion into a topical gel system. Carbopol-940 (0.5 %w/v) in double distilled water was allowed to swell for 12 h. Ciprofloxacin HCl entrapped nanoparticles suspension was dropwise added to swollen carbopol-940 gel and constantly stirred to get homogeneous mixture. Triethanolamine base equivalent to molar ratio 1:1 with carbopol-940 was added to this homogeneous mixture to obtain translucent gel.[20]

Skin irritation test

Skin irritation was evaluated by Draize method without using abraded site. Briefly, Ciprofloxacin HCl entrapped nanoparticles embedded topical gel was applied on shaved and cleaned dorsal surface of rat. After 1,24 and 72h of gel application, the test sites were evaluated for erythema (redness value) and edema by visual score graded as: 0-no reaction, 1-slightly erythema, 2-moderate scaly and erythema, 3-severe scaly with erythema and edema.

Results and Discussion

Experimental design

The formulation was prepared as 9 sets using two variables following 3^2 factorial designs. Ciprofloxacin HCl entrapped chitosan nanoparticles were prepared by Ion-gelation method. Optimized formulations selected by the design expert software were prepared and parameters were compared to the expected values. A full factorial design was used to conduct a comprehensive analysis of the components. The gathered data were statistically analysed using Design Expert software based on the findings of variables on responses. A quadratic model was suggested as the highest order polynomial for entrapment efficiency, where the additional terms are significant and the model is not aliased. ANOVA was used to examine significance of model and fit analysis (Table:1). Influence of

chitosan and sodium tripolyphosphate concentration on entrapment efficiency (Y) was shown to be significant by ANOVA and a quadratic equation 2 was obtained.

$$Y2= +49.85-0.4350*A+6.27*B-3.26*AB-0.6517*A^2-4.28B^2\ldots..2$$

Chitosan and STPP concentrations are denoted by terms A and B, respectively. According to equation 2, the connection between chitosan concentration and entrapment effectiveness is linear and has a positive sign with term A. Entrapment efficiency was shown to improve with increasing chitosan concentration, but as demonstrated by term B, there was an inverse link between STPP concentration and entrapment efficiency, as seen by the negative sign on parameter B. According to fit statistics, the coefficient of determination (R2) for response Y was determined to be 0.998, showing a satisfactory fit between the concentrations of chitosan and STPP on entrapment efficiency. It was noted that the ratio for particle size was discovered to be 189.8.

Optimization studies of Ciprofloxacin HCl entrapped nanoparticles by design expert

In order to identify the values of the independent factors that would result in a particular value of responses, Ciprofloxacin HCl-entrapped nanoparticles were optimised. The desirability approach was used in the numerical optimisation technique. Constraints were introduced to the independent variables to optimise the replies with various values. To maximise trapping effectiveness was the restriction. This constraint was kept common for all the formulations (Table 1.). The Design Expert software produced the suggested optimised formula, which contains 0.25 mg of chitosan and 1 mg of TPP in 100 mL of organic solvent (1% acetic acid), based on the additional constraints. Entrapment effectiveness was assessed in order to validate the experimental model's theoretical prediction. The actual results for entrapment efficiency were discovered to be in very close agreement with the anticipated values, which were validated at 54.96% (Table 1). The estimated relative errors (%) between the predicted and experimental values for the response were found to be less than 5%. The experimental model's validity and predictability were confirmed when it was discovered that the experimental values agreed with the predicted values.

Determination of minimum inhibitory concentration of ciprofloxacin hydrochloride

The MIC of the ciprofloxacin hydrochloride was measured individually and ciprofloxacin hydrochloride loaded nanoparticles were compared against *S. aureus*.

The zone of inhibition of Ciprofloxacin HCl against *S. aureus* in the Figure 9 revealed that as the concentration of Ciprofloxacin hydrochloride increases; results in the increase zone of inhibition. These results showing the minimum concentration of ciprofloxacin hydrochloride inhibiting the bacteria.

The antibacterial activity of ciprofloxacin hydrochloride and ciprofloxacin hydrochloride loaded nanoparticles with 50 mg and 100 mg concentrations were studied against *S. aureus*. The samples containing 50 mg drug loaded nanoparticles and 100 mg drug loaded nanoparticles in Figure 10. revealed that the antibacterial activity of drug loaded nanoparticles were increased as the concentration of drug loaded nanoparticles is increased. This shows higher zone of inhibition when compared with ciprofloxacin hydrochloride alone. Hence, nanoparticles increase the bactericidal activity due to penetration and solubility enhancement property. The zone of inhibition of Ciprofloxacin HCl against *S. aureus* in the Fig.9 revealed that as the concentration of Ciprofloxacin HCl increases; results in the increase zone inhibition. Antibacterial activity of nanoparticles was increased as the concentration of Ciprofloxacin HCl loaded nanoparticles is increased. It shows significantly higher zone of inhibition when compared with Ciprofloxacin HCl alone.

7.2.1 Antibacterial study against *Propionibacterium acne*

The well diffusion method was used to evaluate antibacterial activity. The MIC assay of Ciprofloxacin HCl and Ciprofloxacin HCl loaded nanoparticle suspension was measured against *P. acne*; which was performed (or outsourced) by BioCare Research (India) Pvt Ltd. The zone of inhibition was measured of the varying concentrations of Ciprofloxacin HCl and Ciprofloxacin HCl loaded nanoparticles. From the result observed it was found that as the concentration of Ciprofloxacin HCl increase the antimicrobial activity is also increase. From the result observed it was found that as the concentration of Ciprofloxacin HCl loaded nanoparticle increases the antimicrobial activity also increases. When both the result against p. acne were compared it was found that Ciprofloxacin HCl loaded nanoparticles shows maximum zone of

inhibition at less concentration as compared to plain Ciprofloxacin HCl.

Characterization of Ciprofloxacin HCl entrapped nanoparticles

Particle size and zeta potential measurements

Mean particle size, polydispersity index of Ciprofloxacin HCl entrapped nanoparticles measured by photon correlation spectroscopy are shown in Fig. It was observed that the mean particle size of ciprofloxacin HCl entrapped nanoparticles was found to be 189.8 nm with polydispersity index value 0.365 (Figure 1.) From the results, it can be seen that the prepared Ciprofloxacin HCl entrapped nanoparticles had vesicles in the nano size range, which was also evident from low polydispersity values of the formulation. The lower value of polydispersity index indicated uniformity of vesicle size in the formulation.[21]

Zeta potential is a measurement of the total charges that particles in a given medium have accumulated. It is a crucial characteristic in figuring out the stability of colloidal dispersion. The entire suspension of particles will reject one another if the highest positive zeta potential is above or up to +30mV and the minimum negative zeta potential is above or up to -30mV.[18] The optimised formulation's zeta potential value was found to be slightly inclined towards negative charge (-) 13.0 mV, indicating that a stable colloidal system had been formed (Figure 2).

Entrapment efficiency (%EE)

The capacity of a drug to be encapsulated in nanoparticles is the most important criterion that indicates its loading capability. Entrapment efficiency is affected by factors such as the drug-to-polymer ratio, and speed of homogenizer. Since polymers and entrapment efficiency are directly related, an increase in polymer concentration was found to enhanced percent entrapment efficiency. The percent entrapment efficiency of all the formulation batches were found to be ranged from 41.36 to 54.96 percent (Table 1.), with formulation batch F9 having maximum entrapment efficiency 54.96 percent and the optimized formulation was found to show 54.96 percent entrapment efficiency.

Field Emission Scanning Electron Microscopy study

Particle size data shown that prepared nanoparticles were in nano size range (189.8 nm) (Figure 3.). it was confirmed by field emission scanning electron microscopy (FE-SEM) study. The study was performed to determine three dimensional images which can provide structural and morphological features of optimized formulation. It was observed from FESEM images that the prepared nanoparticles were spherical in shape with smooth surface (Figure 3.).

FT-IR spectroscopy of Ciprofloxacin HCl entrapped nanoparticles

The reports were found to be concurrent with reference spectrum of Ciprofloxacin hydrochloride standard. Fig.4. shows FT-IR spectrum of Ciprofloxacin HCl, Chitosan and Ciprofloxacin HCl entrapped nanoparticles respectively.

Differential scanning calorimetry (DSC)

To gain insight into the phenomenon of Ciprofloxacin HCl entrapped nanoparticle production, DSC investigations were carried out. As shown in Figure 5., in comparison to the DSC thermogram of the drug and polymer, the DSC thermograms of nanoparticles showed complete disappearance of the melting peak of the Ciprofloxacin HCl in the melting temperature range due to formation of nanocarrier system, while a sharp endothermic peak was appeared at 93.80 ^{0}C. This clearly indicates that the drug was completely within the nanoparticles.

Ex-vivo skin diffusion studies

Ex-vivo drug diffusion data were analysed to compare skin diffusion of Ciprofloxacin HCl from developed nanoparticles and to evaluate diffusion. Drug diffusion was found to be faster and higher in formulation as compared to aqueous suspension of drug.

The cumulative diffusion from Ciprofloxacin entrapped nanoparticles and Ciprofloxacin HCl aqueous suspension are shown in Figure 6**.** Cumulative drug diffusion from Ciprofloxacin HCl aqueous suspension was found to be in h and Ciprofloxacin HCl entrapped nanoparticles shown drug diffusion in h. The enhancement in drug diffusion using nanoparticles was found to be as compared to aqueous drug suspension.

Small particle size of Ciprofloxacin HCl entrapped nanoparticles was found to favour permeation across skin. It was observed that permeation rate was faster and more with prepared nanoparticles. Depot formation mechanism was responsible for enhanced permeation of Ciprofloxacin HCl entrapped nanoparticles across the skin.

In-vitro drug release studies

In-vitro release of Ciprofloxacin HCl from nanoparticle loaded gel and Ciprofloxacin HCl loaded gel are as shown in Figure 7., A solvent

blend consisting of phosphate buffer pH 7.4 and ethanol in 70:30 ratio was used as the release media favouring Ciprofloxacin HCl stability and solubility. The drug release from nanoparticles shown slightly higher and prolonged release of about over 24h as compared to Ciprofloxacin HCl gel. (Table 3.)

Skin irritation test

Skin irritation test was performed to evaluate effect of gel formulation on skin after its application for 72h. Results are analysed visually and graded according to Draize method based on skin irritation index.[10] The skin irritation was observed between control, and nanoparticles gel. The positive control shows score 2 type of skin irritation i.e., moderate erythema was observed. (Table 4.) The optimized batch (B3) containing nanoparticles gel does not produce any skin irritation i.e., no erythema or edema was observed in 72hrs. (Figure 8.)

Conclusion

The aim of the present study is to formulate and evaluate Ciprofloxacin HCl loaded nanoparticle gel for the treatment of acne vulgaris. Preformulation studies such as Melting point, solubility, partition coefficient, UV-spectrophotometry, FT-IR, DSC were carried out. The antimicrobial studies were carried out against *S. aureus* and *P. acne* by measuring zone of inhibition. When Ciprofloxacin HCl and Ciprofloxacin HCl loaded nanoparticles against both the species of bacteria, it was found that the Ciprofloxacin HCl loaded nanoparticles shows maximum zone of inhibition. Hence it can be concluded that the nanoparticles preparation shows better antibacterial activity with increase in zone of inhibition. For the preparation of Ciprofloxacin HCl loaded nanoparticles the drug-excipient compatibility studies were performed using FTIR and DSC studies, which do not show any interaction between them. The Ciprofloxacin HCl loaded nanoparticles were prepared by using Ion-gelation method followed by high-speed homogenization. The concentration of Chitosan and STPP varied with the optimized batch for preparation of Ciprofloxacin HCl loaded nanoparticles. The characterization of Ciprofloxacin HCl loaded nanoparticles were done by %EE, particle size, PDI, zeta potential and visual characterization by FE- SEM. Formulation was optimized using 3^2 factorial designs, it was observed that concentration of Chitosan: STPP ratio (0.25:1) was found to be optimum in particle size 189.8nm, %EE, PDI and zeta potential +19.32. The particle size was found to reduce with increase in concentration of STPP, %EE was also increase with increase in concentration STPP, the positive zeta potential was responsible for the stability of the formulation. The optimized batch (F9) of Ciprofloxacin HCl loaded nanoparticles were then formulated into topical gel. Preliminary trials of varying concentrations of Carbopol 940P in concentration 0.5 to 1.5 %w/v were observed with the change in viscosity, spreadability and drug content. It was observed that 1% concentration of Carbopol was found to have viscosity 3500 cP, spreadability 16.37g/cm and drug content 85.7% which were found to be in optimum ranges. The formulated gel was colourless, characteristics in odour with pH 7.308 suited for topical formulation. This shows good homogeneity and extrudability. The produced formulation followed zero order kinetics, according to the release kinetics, and due to the bioadhesive properties of chitosan, a depot was formed that allowed the medication to be released over a period of 12 hours. Therefore, it was determined that the gel made of chitosan nanoparticles loaded with ciprofloxacin HCl enhanced drug penetration and extended release over a 12-hour period. The ex-vivo permeation studies were performed and it was found that highest drug permeation when compared with plain drug solution and marketed formulation. Animal studies were performed and no irritation of Ciprofloxacin HCl loaded nanoparticles was observed upto 72hrs.

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Table 1. List of formulation variables based on the experimental design

Batch	Factor 1 A: chitosan (mg)	Factor 2 B: TPP (mg)	Response 1 Particle size nm	Response 2 Zeta Potential mv	Response 3 EE %
F1	2	0.25	283.74	18.06	41.36
F2	0.25	0.625	219.51	12.02	49.78
F3	1.125	1	150.3	29.21	51.56
F4	2	0.625	213.32	15.36	48.69
F5	1.125	0.625	234.68	16.83	49.78
F6	2	1	155.65	29.87	47.68
F7	1.125	0.25	275.68	22.27	39.65
F8	0.25	0.25	267.79	20.82	35.6
F9	**0.25**	**1**	**189.8**	**19.32**	**54.96**

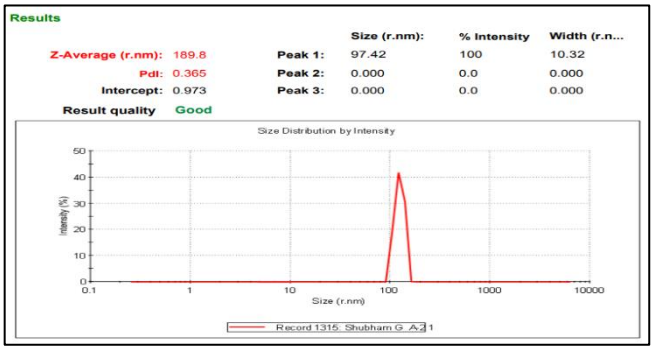


Figure 1. Particle size of optimized batch

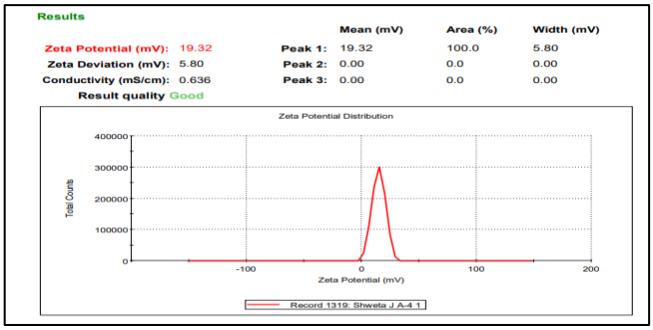
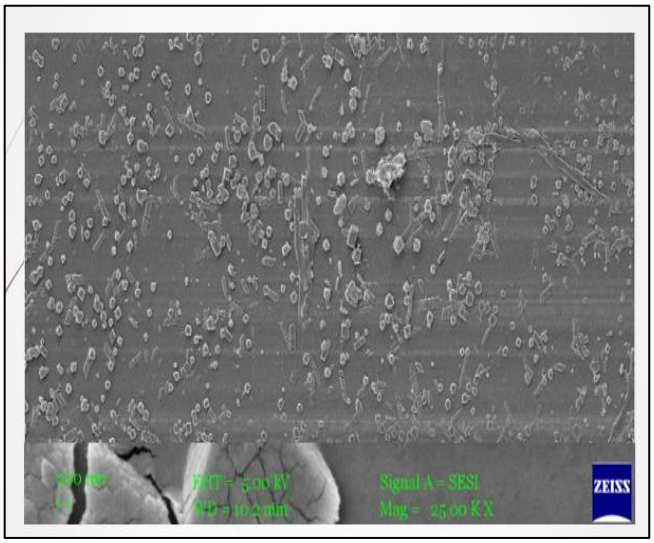


Figure 2. Zeta Potential of Optimized batch



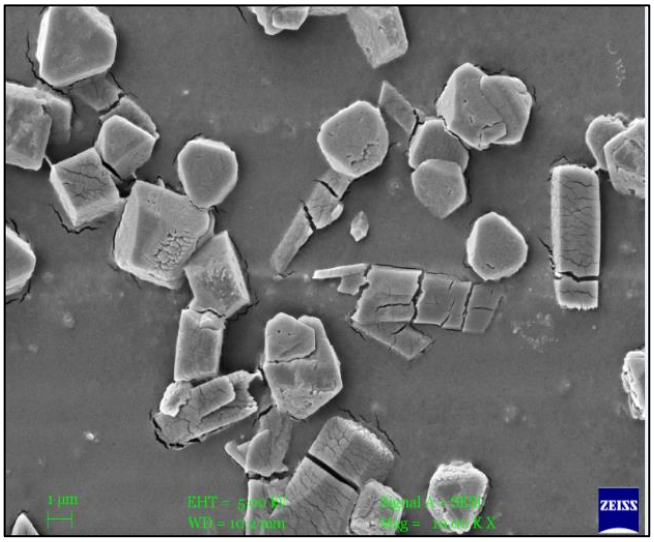


Figure 3. FE-SEM images of Ciprofloxacin HCl loaded nanoparticles

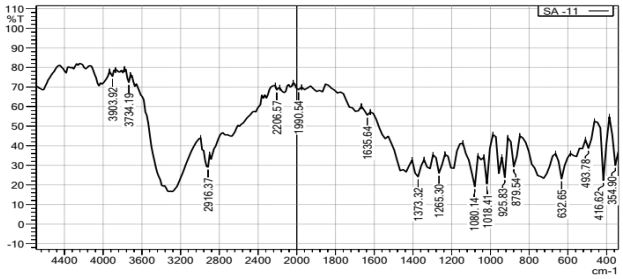


Figure 4. FT-IR spectroscopy of Ciprofloxacin HCl entrapped nanoparticles

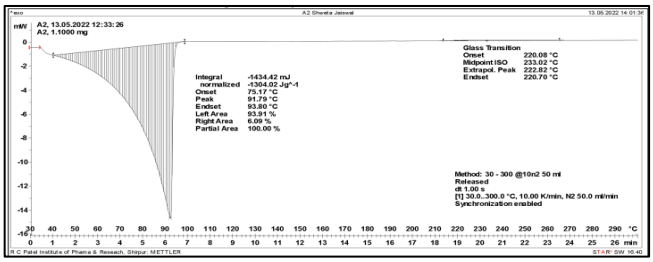


Figure 5. DSC of Ciprofloxacin HCl loaded nanoparticles

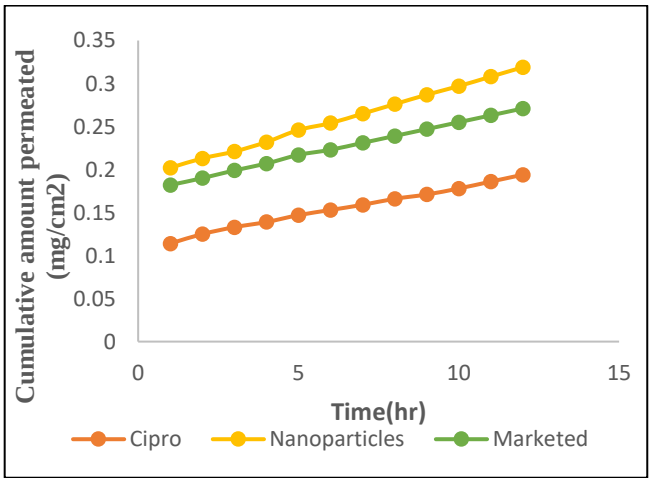


Figure 6. Ex-vivo skin permeation profile comparison

Table 2. Amount of drug permeated and deposited

Formulation	Total amount of drug permeated after 12hr(mcg/cm^2)	Amount of drug deposited in the skin after 12hr (mcg/cm^2)
Plain drug	194.65	18.46
Marketed formulation	271.23	56.79
Drug loaded nano formulation	319.98	96.98

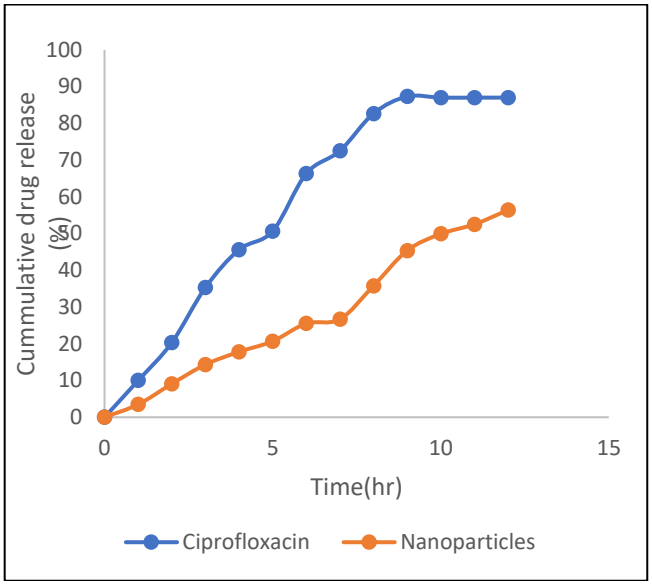


Figure 7. *In-Vitro* drug release profile

Table 3. Drug release kinetic studies

Sr. No.	Model	R^2 Values	
		Nanoparticle suspension	Plain drug solution
1	Zero order	0.9658	0.9743
2	First order	0.8563	0.8436
3	Higuchi	0.7896	0.7986
4	Korsmeyer- Peppas	0.8696	0.8745

Table 4. Acute skin irritation studies

Groups	**Erythema Scores**	
	24 hr	**72 hr**
Group I (Marketed formulation)	0	0
Group II (NaCl 0.9%)	0	0
Group III (C. HCl Nano gel)	0	0
Group IV (1N NaOH)	1	2

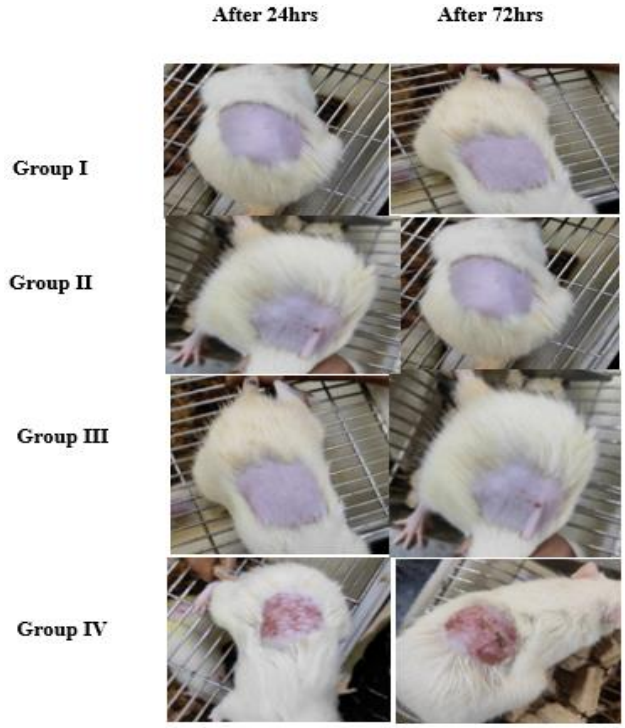


Figure 8. Acute skin irritation studies using rats

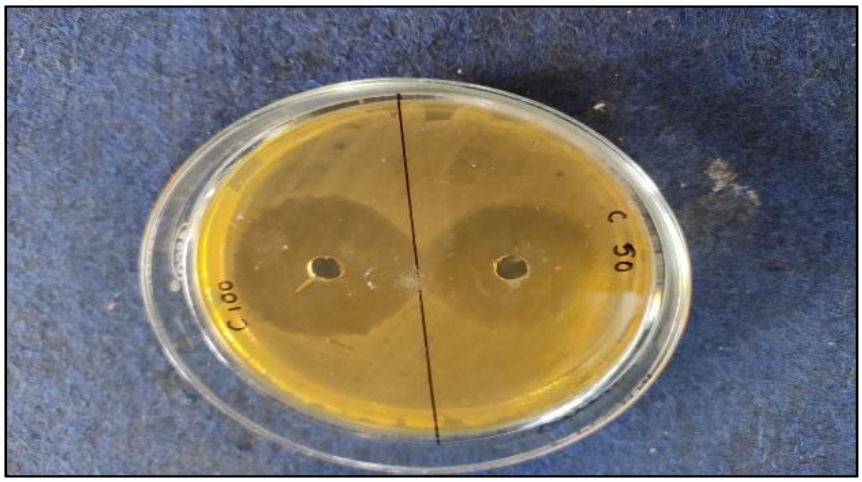


Figure 8. The zone of inhibition of Ciprofloxacin HCl against *S. aureus*

Table 5. Zone of inhibition S. aureus at different concentration of Ciprofloxacin HCl

Concentration (mg/ml)	**S. aureus Zone of inhibition (in mm)**
50	30
100	35

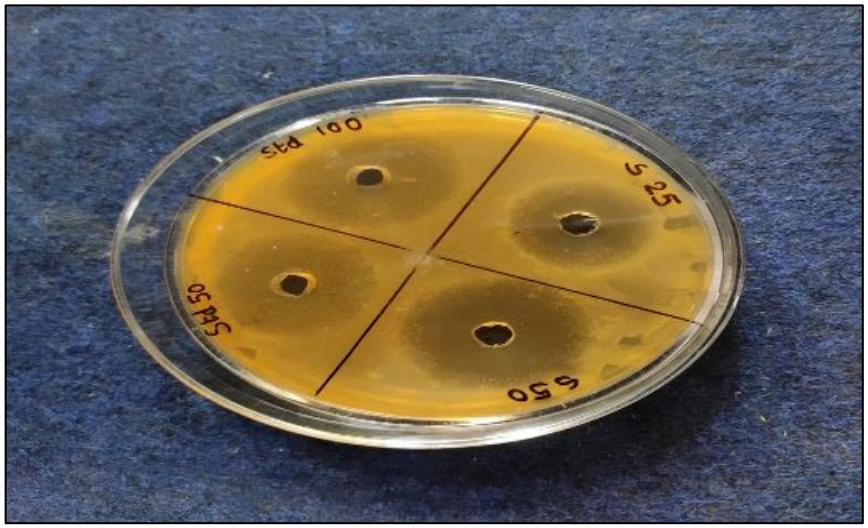


Figure 9. Comparative Antimicrobial activity of *Ciprofloxacin HCl (Std) and Ciprofloxacin HCl loaded nanoparticles suspension (S25 & S50)* against S. aureus

Table 6. Comparative Antimicrobial activity of *Ciprofloxacin HCl (Std) and Ciprofloxacin HCl loaded nanoparticles suspension (S25 & S50)*

Concentration (mg/ml)	**S. aureus Zone of inhibition (in mm)**	
Sample	**Ciprofloxacin HCl**	**Ciprofloxacin HCl loaded nanoparticles**
50	**32**	**36**
100	**35**	**38**

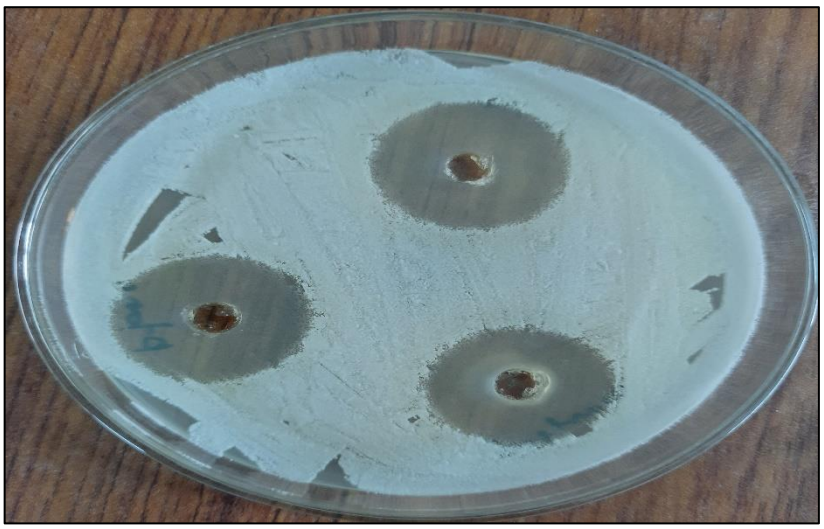


Figure 10. Zone of inhibition of Ciprofloxacin HCl against *P. acne*

Table 7. Zone of inhibition at different concentrations

Concentration (mg/mL)	**P. acne zone of inhibition (in mm)**
100	**15**
150	**17**
200	**20**

Figure 11. Zone of inhibition of Ciprofloxacin HCl loaded nanoparticles

Table 8. Zone of inhibition of Ciprofloxacin HCl loaded nanoparticles against *P. acne*

Concentration mg/mL	P. acne zone of inhibition (in mm)
25	25
50	29
100	35

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