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Formulation optimization and evaluation of Terbinafine hydrochloride nanoparticles for topical applications

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Abstract
Objectives: Aim of the present study was to improve bioavailability and targeted drug delivery. Mucoadhesive nanoparticles of terbinafine hydrochloride had been formulated in order to achieve the prolonged retention at the site of application results in less frequent dosing and sustained response.
Methods: The chitosan nanoparticles of terbinafine Hydrochloride was prepared by modified ionic gelation method and optimized by using Box-Behnken Design. The optimized nanoparticles formulation further characterized for the particle size, percentage drug encapsulation efficiency, percentage yield and mucoadhesive strength. The drug permeation behavior of the optimized formulation was studied by using Franz diffusion cell using goat skin and compared with marketed formulation.
Results and conclusion: The nanoparticles size below 100nm was prepared successfully by modified ionic gelation method. The low flux value of terbinafine HCl mucoadhesive nanoparticles as compared to drug suspension and marketed formulation revealed that the nanoparticles permeated through goat skin mucosa in a controlled manner up to 24 hrs, whereas terbinafine suspension and marketed formulation show high permeability over short duration. Thus topical delivery of terbinafine hydrochloride nanoparticles is an efficient tool to improve its bioavailability and therapeutic effect against fungal infections.

Keywords: Mucoadhesive, nanoparticles, box-behnken design, chitosan, terbinafine hydrochloride, fungal infections

1. Introduction
Terbinafine HCl is FDA approved most widely used antifungal drug for the treatment of various skin infections like athlete's foot, fungal nail infections, ringworm, pityriasis versicolor, jock itch and its broad spectrum activity make it effective against dermatophytes, candida species, molds, yeast, fungi, aspergillus species, and fusarium species. Oral drug delivery of terbinafine HCl suffers with two major disadvantages one is high first pass metabolism and another one is poor aqueous solubility and thus low oral bioavailability [1, 2]. Due to high lipophillic nature terbinafine HCl show good permeation across the epithelial barriers and thus topical drug delivery is a better alternative over its oral delivery to improve bioavailability and drug targeting [3].
Nanoparticles are widely used as drug delivery tool to provide enhanced solubility, better bioavailability, pronounced systemic stability, high drug loading capacity, significant blood circulation time and selective distribution in the organs/tissues with longer residence time [4, 5]. Further, mucoadhesive polymeric nanoparticles increase the amount of drug that reaches the blood circulation by improving its mucosal contact and absorption at the application site. Chitosan is a natural polymer widely used for the fabrication of mucoadhesive nanoparticles owing to its better stability, biocompatibility and low toxicity along with simple formulation methods and versatility in route of administration [6, 7]. Mucoadhesive nanoparticle is an ideal approach for both the controlled release and targeted delivery of drugs.
The main focus of this study is to improve bioavailability and localized drug delivery. Mucoadhesive nanoparticles of terbinafine hydrochloride has been formulated in order to achieve the prolonged retention resulting in less frequent dosing and enhanced therapeutic effect at the target site.

2. Materials and Methods

2.1 Drug and Chemicals

Terbinafine HCl was obtained from Hetero Labs Pvt. Ltd, Hyderabad. Chitosan (CH) was obtained as gift sample from CIFT, Kochi, India. All other chemical and excipients used in the present study were of analytical reagent grade.

2.2 Preparation of Terbinafine HCl loaded chitosan nanoparticles

Chitosan (CH) nanoparticles (Nps) were prepared by modified ionic gelation technique using tri-polyphosphate pentasodium (TPP) as cross linking agent (8). Figure1 represents the schematic representation for the formation of chitosan nanoparticles. After literature review, the chitosan to TPP ratio of 4:1 was selected for the formulation of chitosan nanoparticles [9, 10]. In this method, 0.1%w/v chitosan in 1% acetic acid solution was prepared with whole night stirring on a magnetic stirrer and pH was adjusted to 5.6 using aqueous solution of sodium hydroxide. A constant amount of drug was added to the chitosan solution. TPP aqueous solution was added drop wise to the chitosan solution by continuous stirring for 30 minutes. As a result of ionic cross linking of chitosan with TPP, a slight milky turbid solution was obtained, which was further stirred for half an hour. The nanoparticles dispersion prepared was separated by cooling centrifugation at 13,000 rpm for 1hr. The resultant supernatant was carefully decanted in a separate test tube and the pellets collected from bottom of the tube were redispersed in deionised water (10ml) and sonicated for 2 minutes. 1ml of this solution was further diluted with appropriate volume of deionised water, sonicatedand analyzed for particle size, size distribution and zeta potential. The undiluted nanoparticles after adding 2% D -mannitol (cryoprotectant) were freeze dried using lyophilizer. The freeze dried nanoparticles were collected and stored in air tight container for further studies.

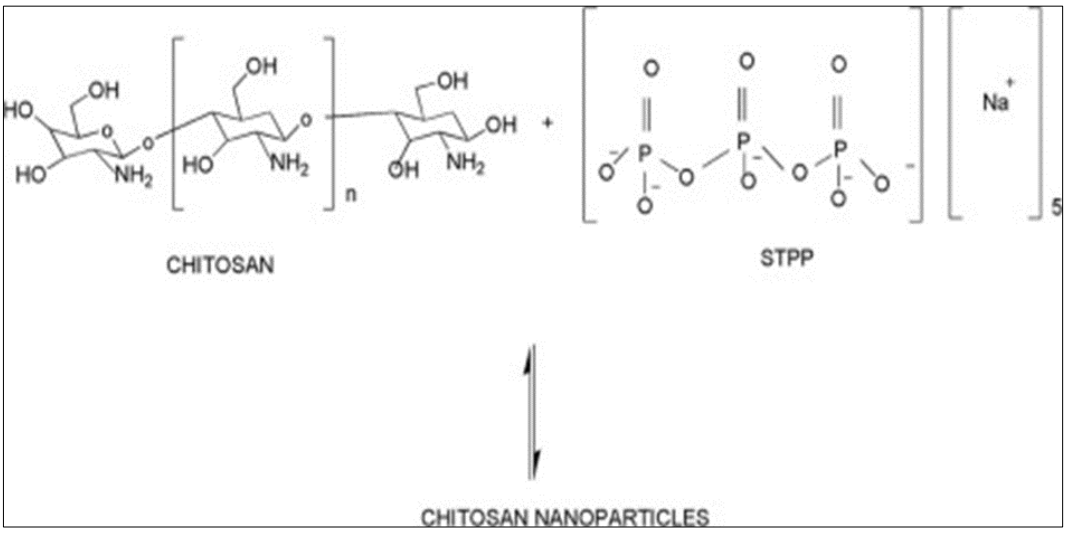

Fig 1: A schematic representation for the formation of chitosan nanoparticles

2.3 Optimization of terbinafine HCl loaded nanoparticles

The formulations of terbinafine HCL loaded nanoparticles were optimized by using Box-Behnken Design (BBD). The effects of selected three numeric factors (amount of polymer (Chitosan), amount of Cross linking agent (TPP) and stirring speed) were studied each at three level on the three responses (particle size, drug entrapment efficiency and percentage yield) were evaluated by Box-Behnken Design in order to create optimum design space (11, 12). The software Design Expert 11 (Stat-ease Inc. Minneapolis, MN) was employed for statistical analysis of the obtained data. The process parameters (with low, medium and high levels) and experimental design layout is illustrated in Table1.

Table 1: Composition of various formulations of terbinafine HCl loaded Chitosan nanoparticles prepared as per experimental design.

		Factor 1	Coded factors Level Factor 2	Factor 3			
Formulation code	Run	X1 Chitosan concentration (%)W/v	X2 TPP (%)W/V	X3 Stirring speed Rpm			
12	1	0	1	1			
10	2	0	1	-1			
5	3	-1	0	-1			
14	4	0	0	0			
9	5	0	-1	-1			
7	6	-1	0	1			
1	7	-1	-1	0			
15	8	0	0	0			
17	9	0	0	0			
3	10	-1	1	0			
2	11	1	-1	0			
16	12	0	0	0			
6	13	1	0	-1			
8	14	1	0	1			
4	15	1	1	0			

13	16	0	0	0			
11	17	0	-1	1			

Details of Process parameters used in the design optimization			
Factors code Coded Levels of Numeric factors	Minimum	Medium	Maximum
X1 Polymer Concentration	-1	0	1
X2 TPP Concentration	-1	0	1
X3 Stirrer Speed	-1	0	1
Polymer			
Chitosan (%)	0.15	0.3	0.45
Cross linking agent			
Sodium Tripolyphosphate (TPP) %	0.03	0.06	0.1

2.4 Characterization of the Terbinafine HCl loaded chitosan nanoparticles

2.4.1 Particle size determination: Average particle size (Z-average), of the formulated nanoparticles were determined by dynamic light scattering analysis using Zetasizer. All the measurements were carried out by dispersing the lyophilized nanoparticles in appropriate volume of deionised water at 25 ◦C. 1 ml sample of the nanoparticles dispersion were placed in disposable cuvettes for particle size analysis. Each sample was analyzed in triplicate.

2.4.2 Drug entrapment efficiency (%DEE): The supernatant of formulations after centrifugation were collected and filtered. The amount of drug present in the supernatant was determined by UV spectrophotometer. A standard calibration curve of concentration versus absorbance was plotted to calculate the amount of drug present in the supernatant. The amount of drug present in supernatant (w) was subtracted from the total amount of drug taken for the preparation of nanoparticles (W) and percentage drug entrapment efficiency (% DEE) was calculated in triplicate manner by using the following formula:

$$\% \text{ DEE} = \frac{\text{Total amount ofdrug(W)} - \text{Free drug in supernatant(w)}}{\text{Total amount ofdrug (W)}} \times 100$$

2.4.3 Percentage (%) yield: % Yield of mucoadhesive nanoparticles was calculated by the following formula:

$$\text{Percentage Yield} = \frac{\text{Weight of nanoparticles}}{\text{Weight of polymer} \times \text{Weight of drug}} \times 100$$

2.4.4 Transmission electron microscopy (TEM): The size and morphological characteristics of the optimized nanoparticles formulation were further confirmed by TEM. The formulated nanoparticles suspension was diluted in HPLC grade water and sonicated for 5 min to produce disaggregation of the particles. The sample was negatively stained with 2% solution of phosphotungstic acid, deposited on a 300 mesh formvar coated grid and then analyzed under HR TEM equipment operated at 200 kV [13, 14].

2.4.5 Fourier transform infrared (FTIR) spectroscopy: FTIR spectroscopic analysis of polymer (chitosan), pure drug and drug loaded Nps was carried out using Fourier transform infrared spectrophotometer scanned in the frequency range of 4000 to 400 cm^{-2}. The pellets were prepared by proper mixing of KBr to the sample and compressed in to pellets by using IR hydraulic press and analyzed [15].

2.4.6 Mucoadhesive potential: The mucoadhesive potential determination of the formulated nanoparticles was carried out by using texture analyzer (TA XT2, Stable Microsystems, UK) (16, 17). For the assessment of mucoadhesive potential, discs were prepared by compressing 200 mg of mucoadhesive nanoparticles by direct compression using single punch hydraulic press (K-Imaya Engineers). The goat skin was obtained immediately after slaughter from the local butchery. The skin were washed with deionized water and placed in normal saline solution at 4 ºC for further studies. The disc was attached to the cylindrical probe (20 mm diameter) by the double sided adhesive tape. The tissue was equilibrated for 15 min at 37.0 ± 0.5 ^{0}C before placing on to the holder stage of the texture analyzer. The probe attached to the disc was loaded with 5 kg weight cell. The test speed was settled at 1mm/s and the probe was moved downwards as to touch the skin for 30s and afterwards the probe was subsequently withdrawn. The maximum force required to separate the probe from the tissue (i.e. maximum detachment force; F_{max}) was detected directly from the texture analyzer and was used to evaluate the mucoadhesive potential of the nanoparticles.

2.5 *Ex- vivo* drug permeation study of the Mucoadhesive nanoparticles by using Franz diffusion cell: The drug permeation behavior of the optimized formulation was studied by using Franz diffusion cell (18, 19). For the *ex- vivo* skin permeation study, goat mucosa (obtained from Slaughter house) was used as biological membrane. The skin was washed with phosphate buffer of pH 6.4 and skin with contact area of 1.55 cm^2 mounted on the receptor compartment of the Franz diffusion cell (diameter 10mm, 15ml volume). The dermal face of the tissue was mounted in contact with the phosphate buffer (pH 6.4). Two experimental sets (in triplicate) were performed keeping the temperature 37±0.5 ºC, at stirring speed of 100 rpm. The terbinafine HCL loaded chitosan Nps were resuspended in 2ml phosphate buffer pH 6.4 and placed on the surface of mucosa mounted on the Franz diffusion cell. 2ml of sample was withdrawn from receptor compartment at different time intervals 0min, 15min, 30min, 1h, 2h, 4h, 6h, 10h, 16h, and 24 hr. To maintain the sink conditions withdrawal sample replaced by equal volume of fresh phosphate buffer. The pure drug aqueous solution was taken as control group. The samples were analyzed by UV- Spectroscopy for the estimation of amount of drug permeated as a function of time. The permeation constant (P) and flux (J) were calculated by using the following formula:

$$\text{Permeation constant(P)} = \frac{\text{Slope} \times \text{Volume ofdonar solution}}{\text{Surface area}}$$

$$\text{Flux(J)} = \text{P} \times \text{Concentration of donar solution}$$

3. Results and Discussion

3.1 Preparation, optimization and characterization of terbinafine loaded chitosan nanoparticles

3.1.1 Particle size, % DEE and % yield determination: All the formulated mucoadhesive Nps were subjected to characterization for Particle size, DEE and production yield. The values of all responses measured (particle size, drug entrapment efficiency (% DEE) and %Yield) are shown in Table 2. Particle size of all the formulations were found to be in the range of 109.5 nm to 979.1 nm, % DEE varied from 31.8% to 83.6% and %Yield varied from 45.7 % to 85.1%.

Table 2: The responses obtained from the trial runs as per optimization design (1 to 17)

Trial Run	Particle Size (nm)	DEE (%)	Yield (%)
1	775.4	68.5	59.1
2	689.6	65	54.1
3	206.1	77.9	80.4
4	507.5	68	53.4
5	478	70.1	61.9
6	109.5	82.9	85.1
7	203.8	70.4	77.5
8	490	59.9	48.9
9	559.5	49.8	45.7
10	189.7	83.6	82.3
11	690.7	47	48.9
12	570.6	67.2	55.9
13	608.3	43.7	52.8
14	894	37.5	45.8
15	979.1	31.8	48.1
16	403.7	71.5	63.8
17	505	61.6	57.9

3.1.2 Optimization by Box Behnken Design

Design expert software version 11 was used for the design optimization of the mucoadhesive nanoparticles using Box Behnken Design (BBD) by response surface methodology (RSM) [20]. Total 17 confirmatory runs were suggested by BBD for the optimization of terbinafine HCl loaded chitosan nanoparticles by selecting the three numeric factors (Polymer concentration, TPP concentration and Stirring speed). The objective of optimization was to minimize the particle size, maximize entrapment efficiency and production yield by varying the concentration of polymer and cross linking agent and stirring speed for the preparation desired formulation.

A design space with desirability of 0.982 was generated from the numerical optimization. The optimum formulation parameters, (as suggested by the software) were 0.15% chitosan, 0,886 rpm stirrer speed and 0.321% sodium triployphosphate as the crosslinking agent. The optimized formulation was fabricated in triplicate and evaluated. The numerical optimization was validated by comparing the predicted and actual values of the responses which revealed a statistically insignificant percent prediction error. Optimized formulation of the terbinafine nanoparticles was selected from the design space suggested by the design expert software which was further evaluated for physicochemical, morphological, and in-vivo drug permeation studies through goat skin.

ANOVA for Quadratic model was found to be best fit model for the responses; particle size, production yield and linear model for the response % DDE with p values for X1, X2 and X3 are less 0.0001 and F values as 29.80, 18.01 and 9.58 respectively. P-values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. The adequate precision was the measure of the signal to noise ratio and its value being more than 4 for all these selected response variables and hence model can be used to navigate the optimum design space. The reliability of regression models was also established from the high R^2 for the particle size, drug entrapment efficiency and production yield (0.9746, 0.8060 and 0.9249) values and their similar adjusted R^2 values (0.9419, 0.7613 and 0.8283) respectively. The Lack of Fit F-value for all the three responses was found to be not significant relative to the pure error. Non-significant lack of fit is good if we want the model to fit. Polynomial coded equations were generated for various responses for optimization terbinafine HCl nanoparticles are depicted in Table 3.

Table 3: The following polynomial coded equations were generated for all the Responses

Particle Size		% DEE		% Yield	
+506.26		+62.14118		+53.54000	
+307.88	A	-19.35000	A	-16.21250	A
+94.54	B	-0.025000	B	-0.325000	B
+37.74	C	-0.775000	C	-0.162500	C
+75.62	AB			-1.40000	AB
+95.58	AC			-2.92500	AC
+14.70	BC			+2.25000	BC
-73.98	A^2			+9.21750	A2
+83.54	B^2			+1.44250	B2
+22.20	C^2			+3.26750	C2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Response surface analysis

A. Effect of numerical factors on particle size of Terbinafine HCl Nps: 3D Response surface for the of polymer concentration, cross linking agent (TPP) concentration and stirring speed on particle size of terbinafine HCl nanoparticles are shown in Figure 2 & 5. It was found that the particle size increased with increase in the polymer concentration. At medium level of crosslinking agent, particles size of nanoparticles was found to be minimum. Particle size was found to be increased at higher polymer TPP ratio, due to increase in cross linking agent (TPP) concentration results in to the higher cross linking with the polymer. The stirrer speed at 1000 rpm (maximum), minimum particle size was obtained whereas at lower speed (i.e. 500 rpm) the particle size gets increases. This behavior can be attributed to the fact that low stirring speed did not provide sufficient attrition to nanoparticles whereas high stirring speed provides sufficient attrition. In general particle size was increased with increase in polymer (chitosan) concentration

and decreased with the increase in the crosslinking agent (TPP) concentration and stirring speed.

B. Effect of numerical factors on % DEE of Terbinafine HCl Nps: 3D Response surface for the of polymer concentration, cross linking agent (TPP) concentration and stirring speed on % DDE of terbinafine HCl nanoparticles are shown in Figure 3 & 6. It was observed that DEE increased with decrease in polymer (chitosan) concentration as higher amount of polymer can entrap drug in nanoparticles which may leach out in to the solution. Increase in TPP concentration, increases the cross linking due to more drug entrapped in the particles. At maximum level of TPP concentration the % DDE was found to be maximum 83.6%. With increase in the stirring speed %DEE was also get increased, with maximum level of stirring speed the DDE was found maximum (83.6 %).

C. Effect of numerical factors on production yield of Terbinafine HCl Nps: 3D and 2D Response surface for the of polymer concentration, cross linking agent (TPP) concentration and stirring speed on production yield of terbinafine HCl nanoparticles are shown in Figure 4 &7. It was observed that production yield increased with decrease in the polymer (chitosan) concentration as the entire polymer was converted in to small size of nanoparticles with larger surface area. Medium level of cross linking agent (TPP) resulted in to the high production yield 85.1% and both high and low level of TPP decreased the production yield as low as 48.9% and 48.1% respectively. Maximum level of stirring speed produced high production yield (85.1%).

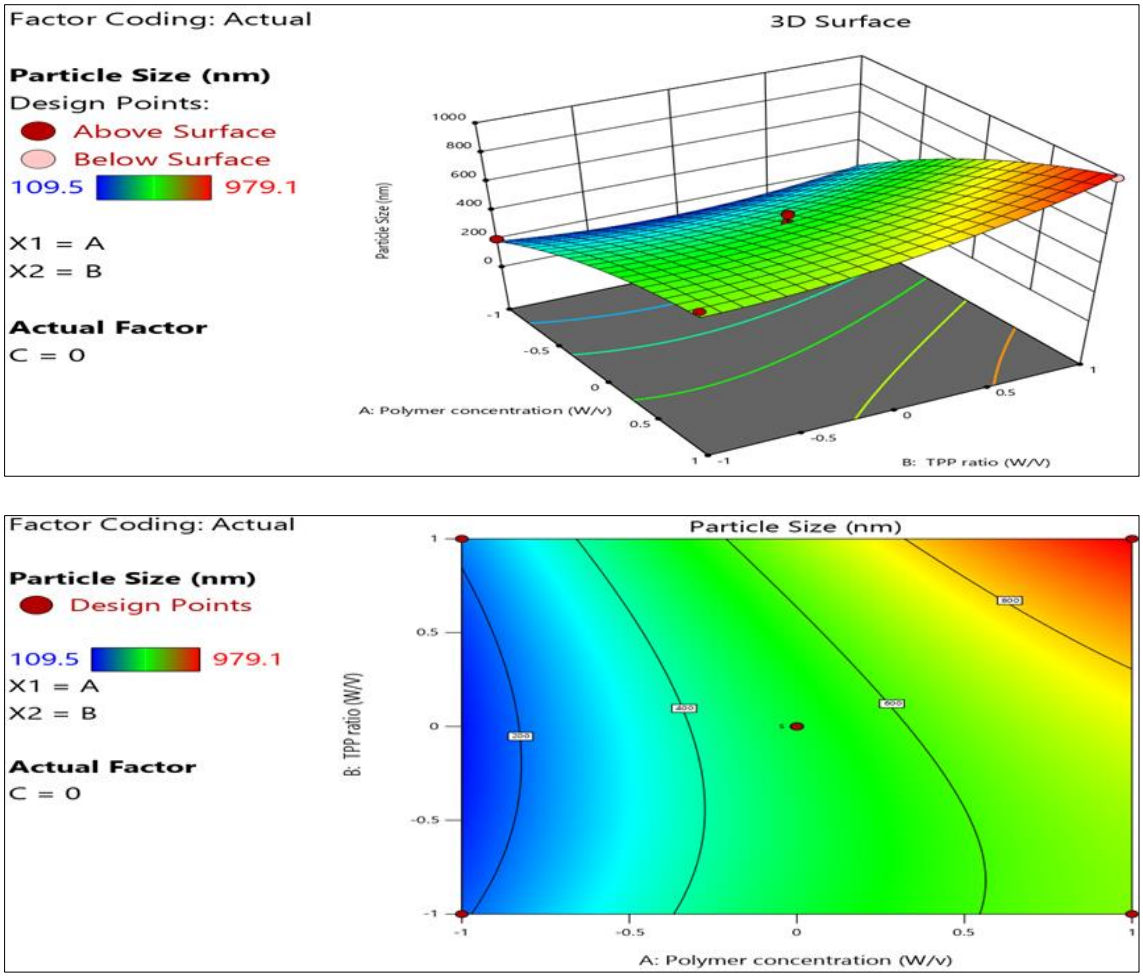

Fig 2: 3D and 2D response surface graphs for particle size

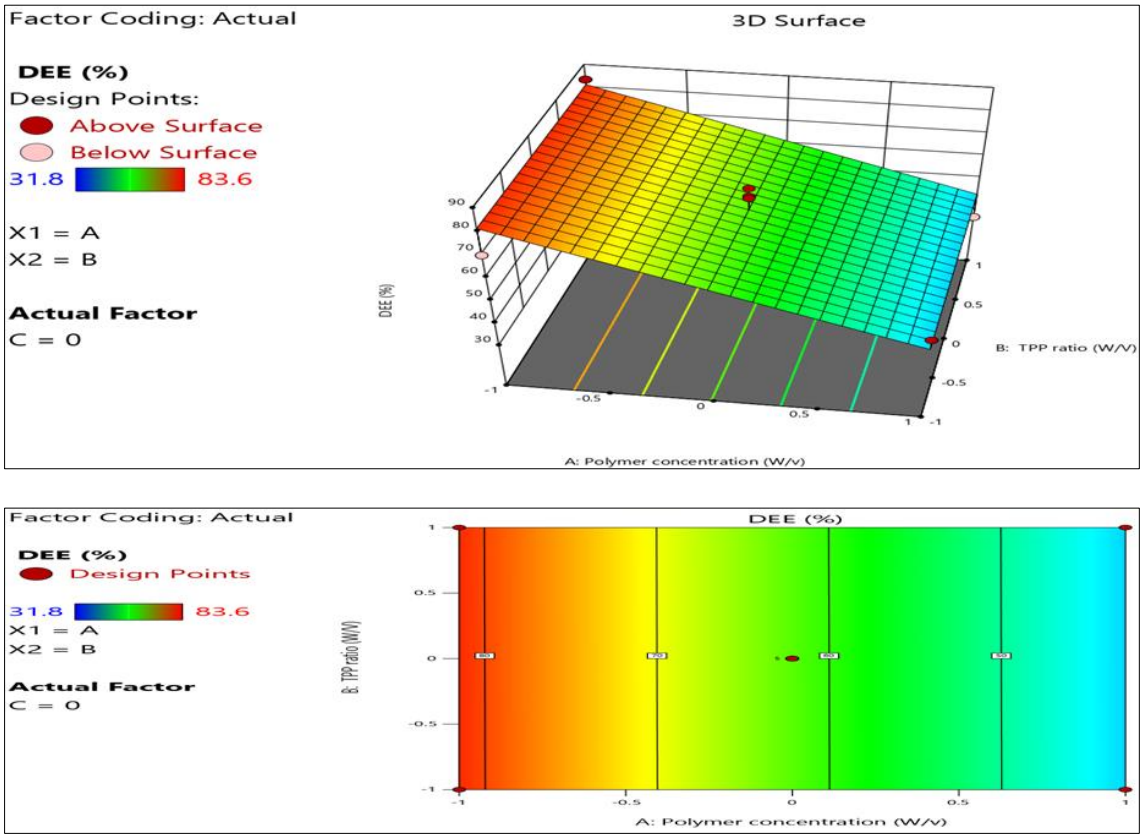

Fig 3: 3D and 2D response surface graphs for %DDE

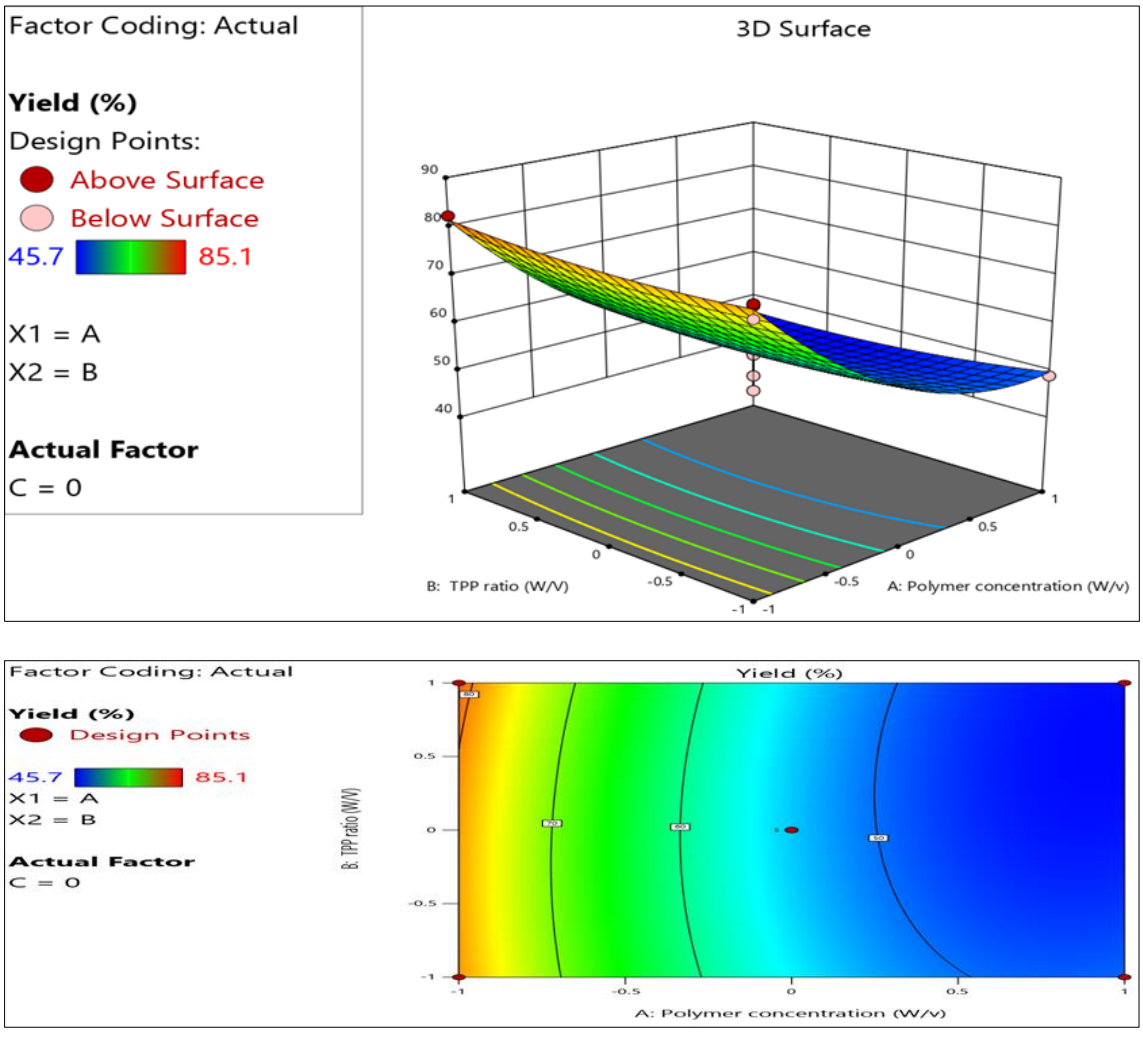

Fig 4: 3D and 2D response surface graphs for Percentage Yield

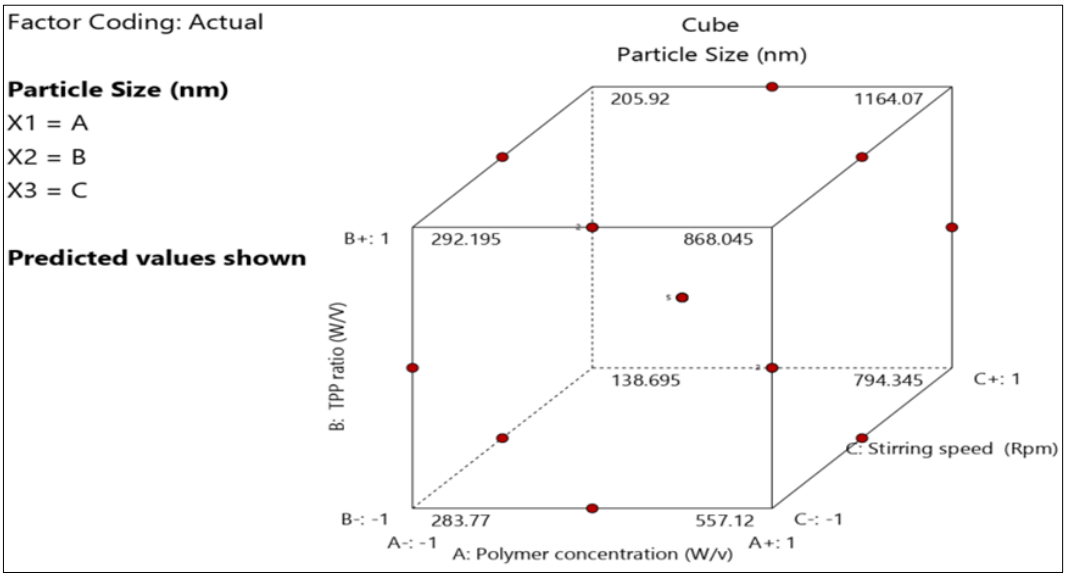

Fig 5: Cube plots showing the effect of X1 (polymer Concentration), (X2) TPP ratio (X3) stirring speed on the particle size of the Terbinafine HCl Nps

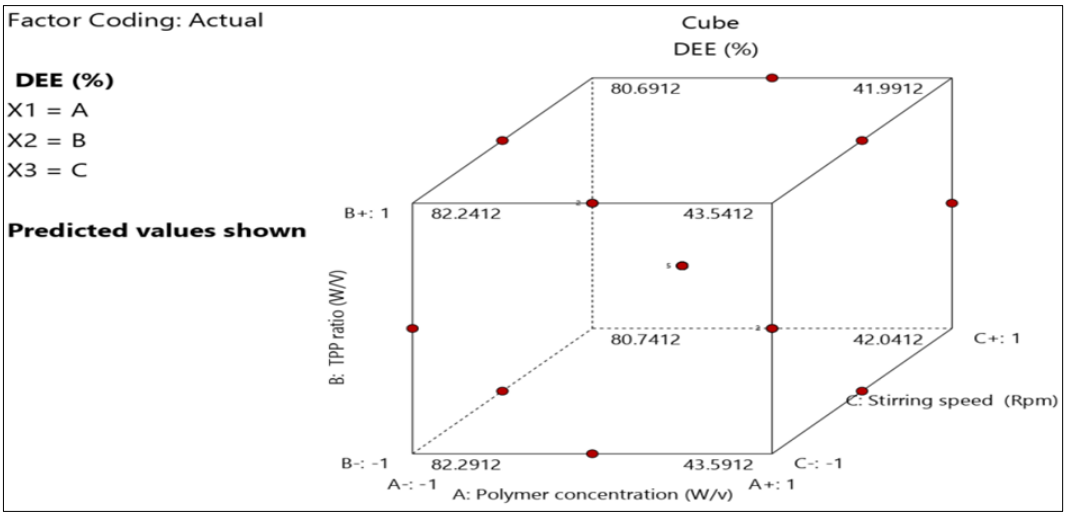

Fig 6: Cube plots showing the effect of X1 (polymer Concentration), (X2) TPP ratio and (X3) stirring speed on the % DDE of the Terbinafine HCl Nps

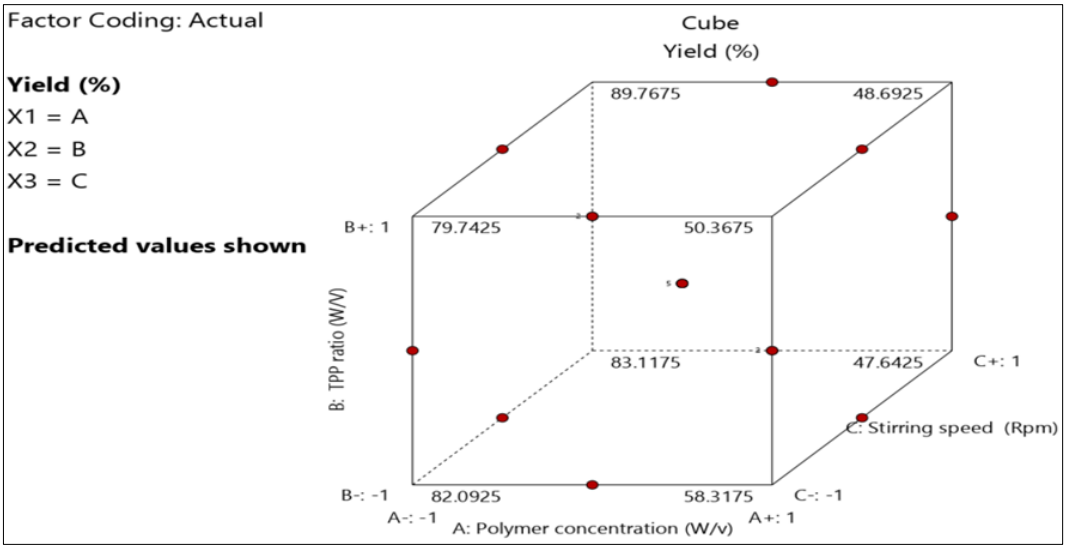

Fig 7: Cube plots showing the effect of X1 (polymer Concentration), (X2) TPP ratio and (X3) stirring speed on the % Yield of the Terbinafine HCl Nps

Desirability: A design space with desirability was generated from the numerical optimization shown in Table 4.

Table 4: A design space with maximum desirability was generated from the numerical optimization

Polymer concentration	TPP conc.	Stirring speed	Particle Size	DEE	Yield	Desirability	
0.15	0.321	0.886	109.456	80.797	85.113	0.982	Selected

A design space with desirability of 0.982 was generated from the numerical optimization. The optimum formulation parameters (as suggested by the software) were 0.15% chitosan, 0,886 rpm stirrer speed and 0.321% sodium triployphosphate as the crosslinking agent. The optimized formulation was fabricated in triplicate and evaluated. The numerical optimization was validated by comparing the predicted and actual values of responses which revealed a statistically insignificant percent prediction error. Optimized formulation of terbinafine nanoparticles was selected from the design space suggested by the design expert software which was further evaluated for the physicochemical, morphological, and *in-vivo* drug permeation studies through the goat skin.

3.1.3 Characterization of the optimized formulation of terbinafine HCl nanoparticles

Particle size, DEE and percentage yield: The optimized formulation was fabricated and evaluated in triplicate. The values of particle size, %DEE and % yield for the optimized terbinafine Nps are depicted in Table 5. Particle size distribution of the optimized formulation is depicted in Figure 8 (21, 22).

Table 5: Particle size, %DEE and percentage yield for the optimized formulation of terbinafine HCl nanoparticles

Sr. No.	Particle Size (nm)	% DEE	%Yield
I	123.3	88.05	83.09
II	130.2	81.00	85.00
III	147.0	78.65	84.45

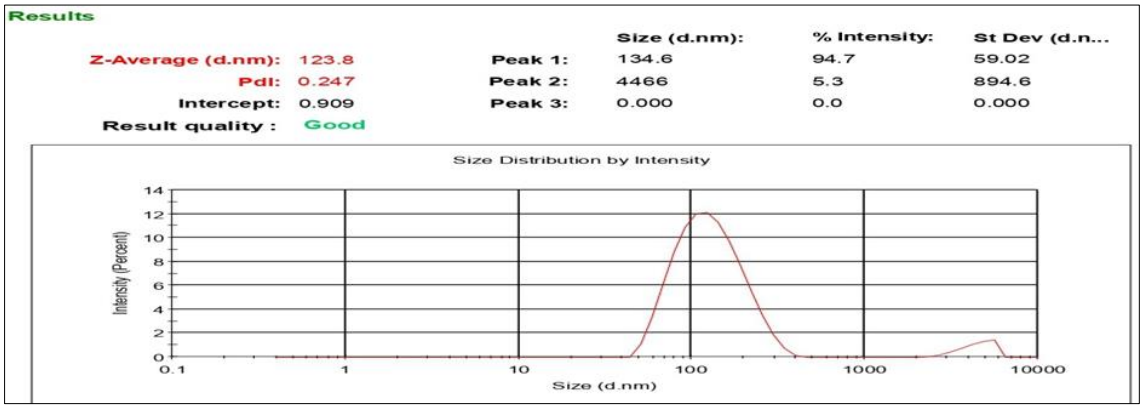

Fig 8: Average Particle size of the optimized formulation of terbinafine HCl nanoparticles

3.1.4 Mucoadhesive strength: Mucoadhesive strength (F_{max}) was measured by the texture analyzer and found to be 0.3N for the optimized mucoadhesive nanoparticles (23, 24). The maximum force required to separate the probe from the tissue was (i.e. maximum detachment force; F_{max}) given by force versus time curve for optimized mucoadhesive nanoparticles formulation is shown in Figure 9.

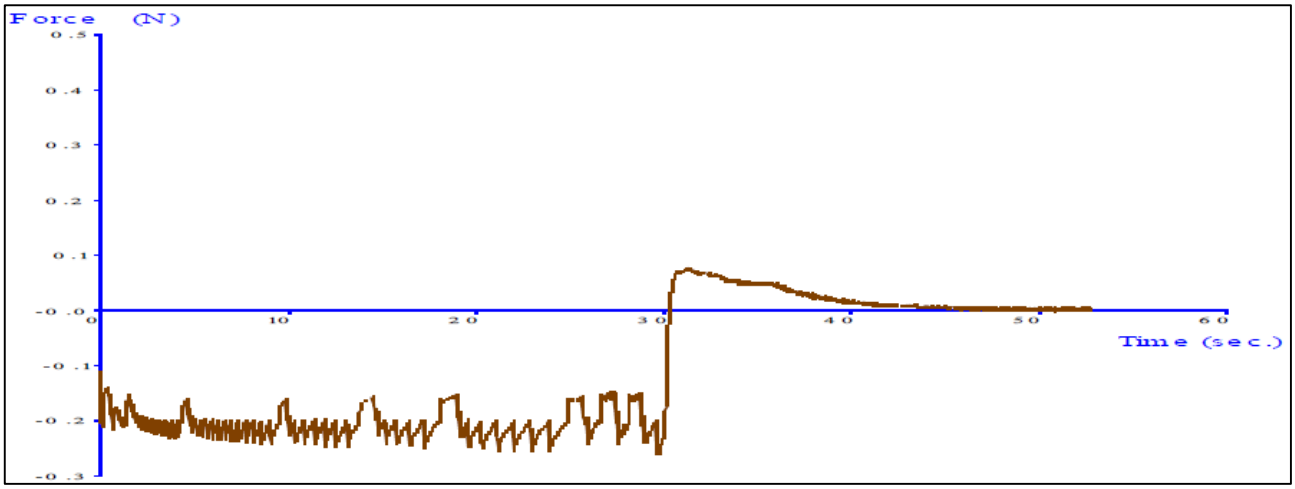

Fig 9: The graph showing force (Y-axis) time (X-axis) curve for the optimized formulation of terbinafine HCl nanoparticles

3.1.5 Transmission Electron Microscopy (TEM): Transmission Electron Microscopy of the optimized formulation of Terbinafine nanoparticles is shown in Figure 10. TEM image shows spherical and smooth shape of chitosan nanoparticles having size range below 100 nm. The particle size found in TEM analysis was less than that analyzed by Zetasizer due to hydrodynamic layer covering the particles got removed during slide preparation in TEM examination and it gives an actual size of chitosan Nps in dry state (25, 26).

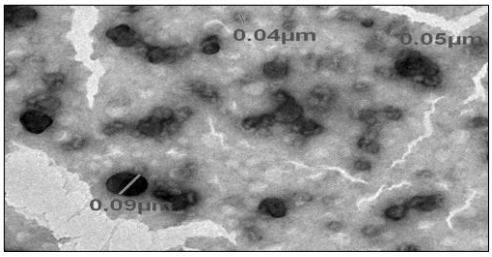

Fig 10: TEM image of the optimized formulation of terbinafine HCl nanoparticles

3.1.6 Ftir analysis

The optimized formulation was characterized by infrared spectroscopy to confirm the successful drug entrapment in the nanoparticles. FTIR spectra of pure drug terbinafine HCl, Polymer chitosan and optimized formulation mucoadhesive Nps are shown in Figure 11, 12 and 13 respectively (27). The FTIR spectra of the optimized mucoadhesive Nps shows the presence all the characteristic peak of the drug as well as polymer, thereby confirming the absence of any chemical interaction between drug and polymer.

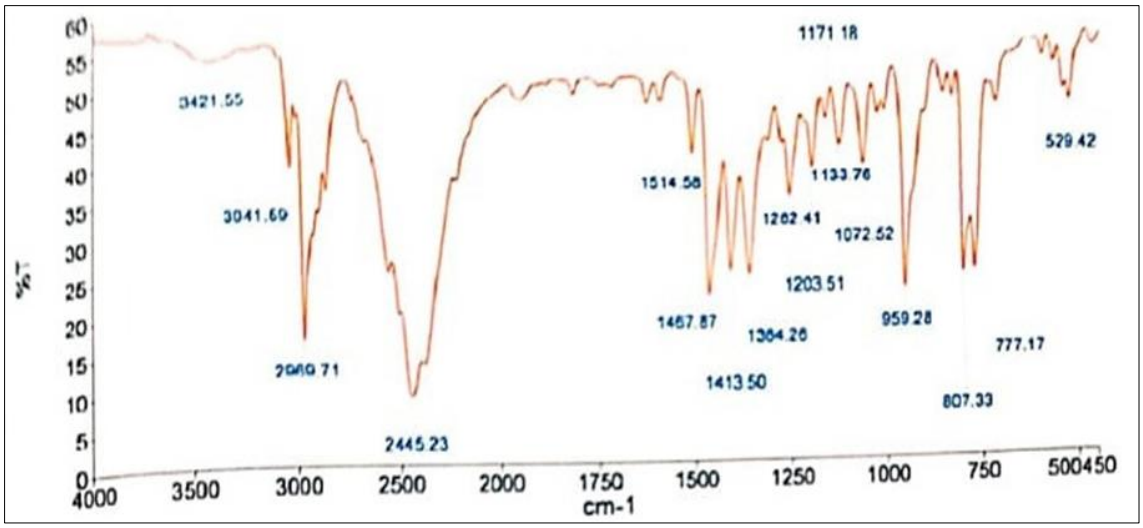

Fig 11: FTIR spectra of the Terbinafine HCl

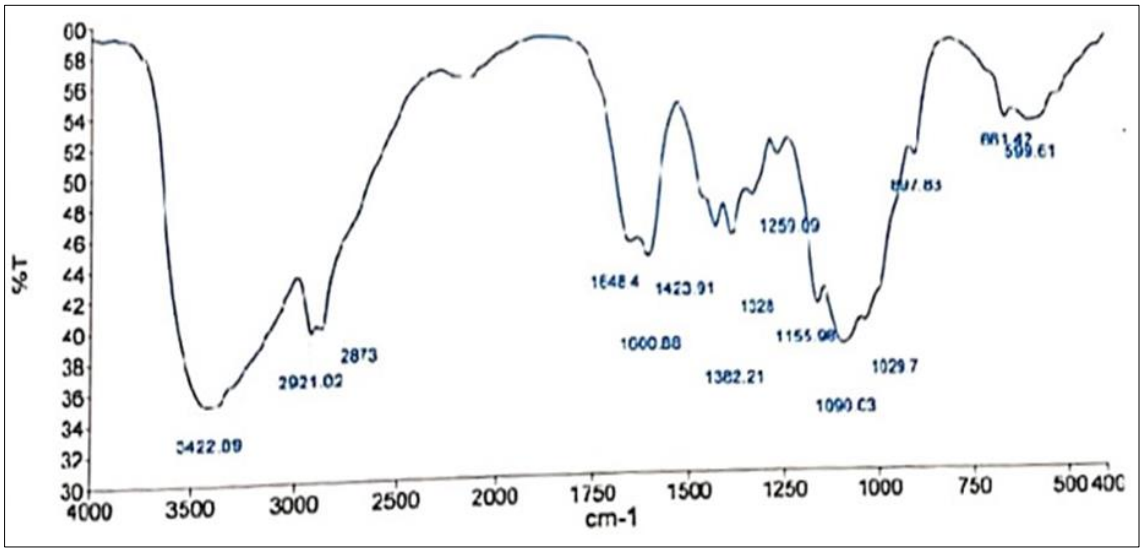

Fig 12: FTIR spectra of the Polymer chitosan

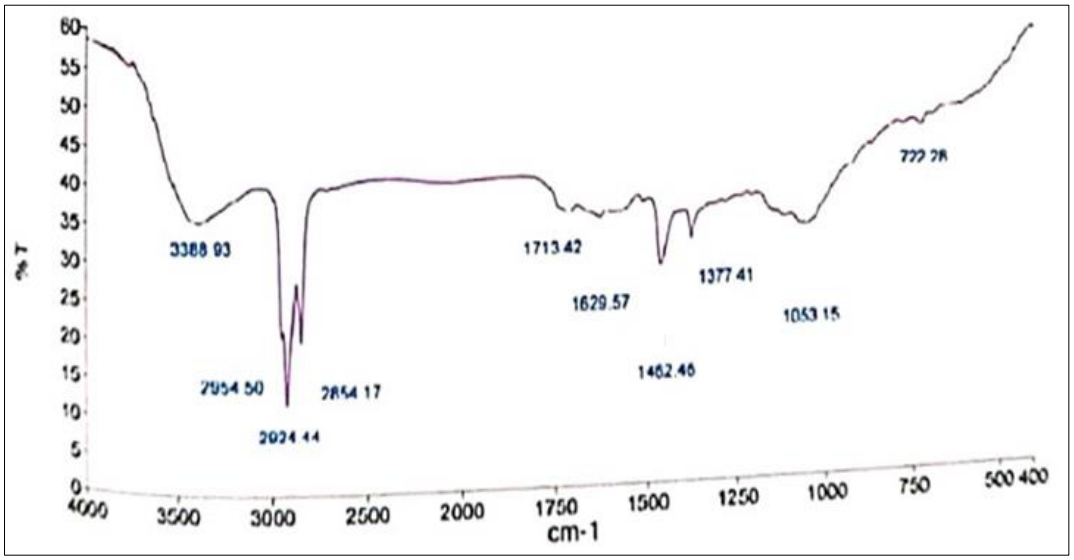

Fig 13: FTIR spectra of the optimized formulation of terbinafine HCl nanoparticles formulation

3.2 *Ex -vivo* drug permeation studies

The goat skin mucosa was selected as biological membrane for the permeation studies by using Franz diffusion cell. The formulated nanoparticles was suspended in carbopol gel for the permeation studies. The terbinafine HCl nanoparticles containing carbopol gel, drug suspension and marketed formulation of terbinafine HCl Terbicep® (1%) were compared for the drug permeation across the epithelial skin layer. The amount of drug permeated at different time intervals are given in Table 6. Drug permeated from the optimized Nps formulation showed sustained release up to 24 hours whereas pure drug suspension showed rapid drug up to 92.6% in 6 hours through goat epithelial tissues. The graph of the % drug permeated versus time is shown in Figure 14. The regression plot was used to calculate the permeation constant given by the slope of the regression line and flux (J) calculated for optimized terbinafine Nps, Drug suspension and Terbicep® 27.97, 34.24 and 37.05 respectively as shown in Figure 15. The higher flux value for the pure drug suspension and Terbicep® as compared to the optimized nanoparticle formulation through the goat skin reveals the fast permeation of the drug whereas drug from the nanoparticles was slowly permeated over longer time period. Thus the nanoparticles permeation behavior follows the sustained drug release pattern.

Table 6: Ex- vivo drug permeation profile for the optimized nanoparticles, drug suspension and marketed formulation of the terbinafine HCl

	Percentage drug permeated		
Time (Hours)	**Optimized Nps**	**Drug suspension**	**Terbicep®**
0	0.0	0.0	0.0
0.25	8.7	8.2	7.5
0.5	17.4	9.5	15.4
1	20.5	38.5	32.6
2	29.9	60.1	52.0
4	41.5	84.2	70.9
6	47.9	92.6	80.5
10	54.2	93.2	88.0
16	62.1	91	93.1
24	73.9	90.5	91.7

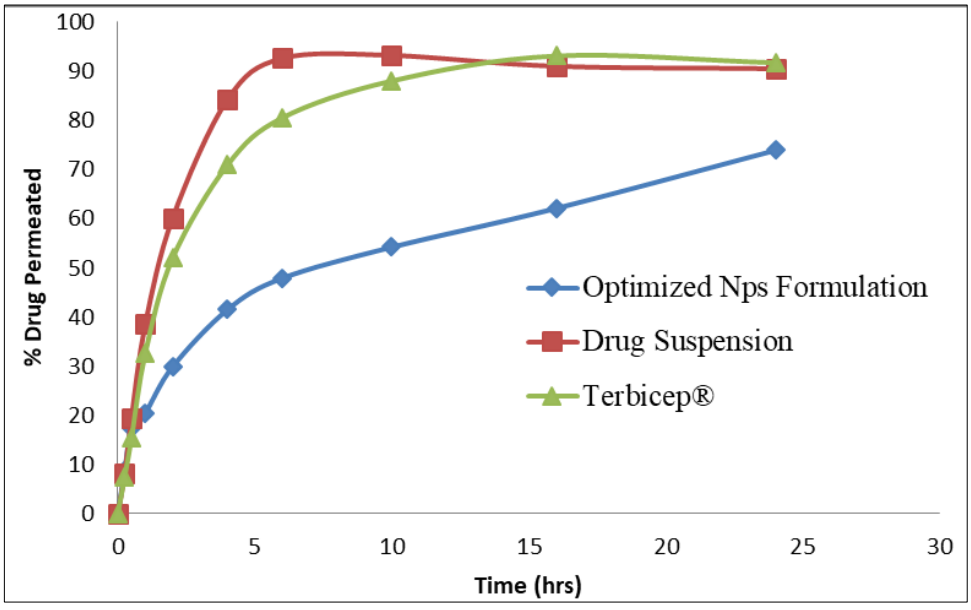

Fig 14: The drug permeation profile showing % drug permeated versus time for the optimized Nps, Drug suspension and marketed formulation (Terbicep®) of the terbinafine HCl

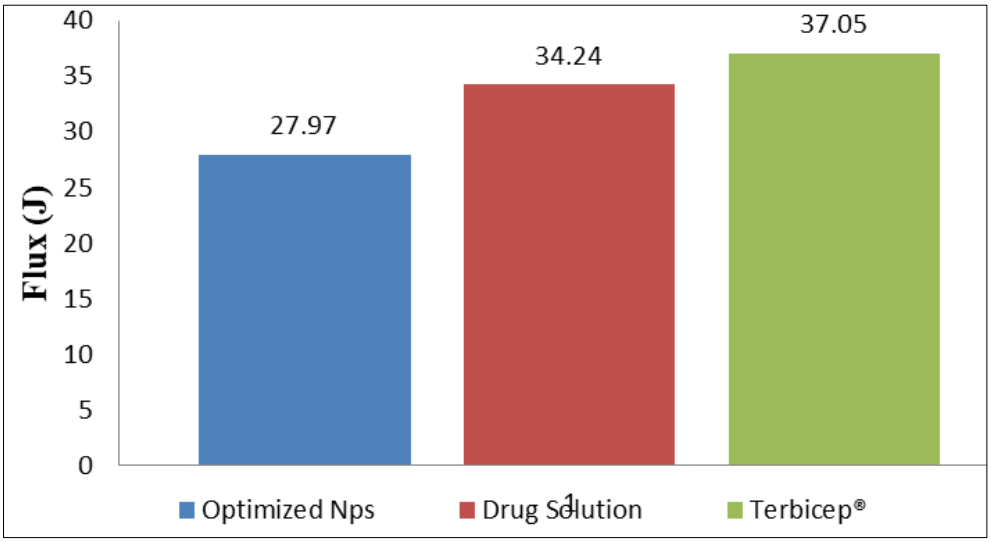

Fig 15: The flux values comparing the drug permeation across for optimized Nps, Drug suspension and marketed of formulation (Terbicep®) of terbinafine HCl

4. Conclusion

The terbinafine HCl nanoparticles were formulated by modified ionic gelation method using chitosan as mucoadhesive polymer and optimized by the Box Behnken Design (BBD). The optimized drug loaded nanoparticles was further characterized and evaluated. The transmission electron microscopic examination reveals the smooth & spherical shape nanoparticles with size range below 100nm. Mucoadhesive strength was found to be appropriate (0.3N approximately).

Ex– vivo skin permeation study using goat skin mucosa was revealed the better permeability up to 24 hrs by terbinafine loaded optimized mucoadhesive nanoparticles formulation as compared to its drug suspension and its marketed formulation Terbicep®. Overall, the topical drug delivery in form of nanoparticles is a better option for the improving bioavailability and sustained effect of poorly water soluble drugs.

5. References

1. Kaur IP, Kakkar S. Topical delivery of antifungal agents. Expert Opin Drug Deliv 2010;7(11):1303-1327.
2. Van der Lubben IM, Verhoef JC, Borchard G, Junginger HE. Eur J Pharm Sci 2001;14:201-207.
3. Gaba B, Fazil M, Khan S, Ali S, Baboota S, Ali J. Nanostructured lipid carrier system for topical delivery of terbinafine hydrochloride. Bulletin of Faculty of Pharmacy 2015;53(2):147-159.
4. Singla AK, Chawla M. Chitosan: some pharmaceutical and biological aspects-an update. J Pharm Pharmacol 2001;53(8):1047-1067.
5. Girotra P, Singh SK. Chitosan: An emanating polymeric carrier for drug delivery, in: Thakur V K & Thakur M K (Eds.), Handbook of Polymers for Pharmaceutical Technologies. Wiley– Scrivener Publishing LLC 2015, 33-60.
6. Syed AA, Rizvi A, Ayman M, Saleh B. Applications of nanoparticle systems in drug delivery technology. Saudi Pharmaceutical Journal 2018;26:64-70.
7. Kumar S, Dilbaghi N, Saharan R, Bhanjana G. Nanotechnology as emerging tool for enhancing solubility of poorly water-soluble drugs. Bionanoscience 2012;2(4):227-50.
8. Prabaharan M, Mano JF. Chitosan-based particles as controlled drug delivery systems. Drug Deliv 2005;12:41-57.
9. Janes KA, Calvo P, Alonso MJ. Polysaccharide colloidal particles as delivery systems for macromolecules. Adv Drug Deliv Rev 2001;47:83-97.
10. Terbojevich M, Muzzarelli RAA. Chitosan, Handbook of Hydrocolloids. In: Phillips GO, Williams PA, editors. Cambridge: CRC Press 2000, 367-376.
11. Ferreira SC, Bruns *et al.* Box Benhnken design: an alternative for the optimization of analytical methods. Analytica Chimica Acta 2007;597(2):179-186.
12. Box GE, Benhnken DW. Some new three level designs for the study of quantitative variables. Technometrics 1960;2(4):455-475.
13. Labhasetwar V, Song C, Levy RL. Nanoparticle drug delivery system for restenosis. Adv Drug Deliv Rev 1997;24:63-85.
14. Nagarwal RC, Kumar R, Dhanawat M, Pandit JK. Modified PLA nano in situ gel: a potential ophthalmic drug delivery system. Colloids Surf B 2011;86:28-34.
15. Vimal G, Taju KSN, Nambi S *et al.* Synthesis and characterization of CS/TPP nanoparticles for oral delivery of gene in fish. Aquaculture 2012;358:14-22.
16. Tobyn MJ, Johnson JR, Dettmar PW. Factors affecting *in vitro* gastric mucoadhesion I. Test conditions and instrumental parameters. Eur J Pharm Biopharm 1995;41:235-241.
17. Woertz C, Preis M, Breitkreutz J, Kleinebudde P. Assessment of test methods evaluating mucoadhesive polymers and dosage forms: An overview. Eur J Pharm Biopharm 2013;85:843-853.
18. Langoth N, Guggi D, Pinter Y. Andreas BS. Thiolated chitosan: *in vitro* evaluation of its permeation properties. Journal of Control Release 2004;94:177-186.
19. Barbero AM, Frasch HF. Pig and guinea pig skin as surrogates for human *in vitro* penetration studies: a quantitative review. Toxicol *in vitro* 2009;23(1):1-13.
20. Sunena Mishra DN, Singh SK, Kumar A. Formulation and optimization of mucoadhesive galantamine loaded nanoparticles. Der Pharmacia Lettre 2016;8(10):206-212.
21. Zheng T, Bott S, Huo Q. Techniques for Accurate Sizing of Gold Nanoparticles Using Dynamic Light Scattering

with Particular Application to Chemical and Biological Sensing Based on Aggregate Formation. ACS Applied Materials & Interfaces 2016;8(33):21585-21594.
22. Saremi S, Atyabi F, Akhlaghi SP, Ostad SN, Dinarvand R. Thiolated chitosan nanoparticles for enhancing oral absorption of docetaxel: preparation, *in vitro* and ex vivo evaluation. Int J Nanomedicine 2011;6:119-128.
23. Lee CA, Kim BS, Cho CW. Quantitative evaluation of mucoadhesive polymers to compare the mucoadhesion. Journal of Pharmaceutical Investigation 2016;46:189-194.
24. Thirawong N, Nunthanid J, Puttipipatkhachorn S, Sriamornsak P. Mucoadhesive properties of various pectins on gastrointestinal mucosa: an *in vitro* evaluation using texture analyzer. Eur J Pharm Biopharm 2007;67(1):132-40.
25. Langoth N, Guggi D, Pinter Y. Andreas BS. Thiolated chitosan: *in vitro* evaluation of its permeation properties, Journal of Control Release 2004;94:177-186.
26. Barbero AM, Frasch HF. Pig and guinea pig skin as surrogates for human *in vitro* penetration studies: a quantitative review, Toxicol *in vitro* 2009;23(1):1-13.
27. Sudhakar Beeravelli, Ravi Varma JN, Ramana Murthy KV. Formulation, Characterization and ex vivo Studies of Terbinafine HCl Liposomes for Cutaneous Delivery. Current Drug Delivery 2014;11(4):521-530.