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Research Article

DEVELOPMENT AND EVALUATION OF FINASTERIDE-LOADED NANOPARTICLES FOR POTENTIAL TREATMENT OF ALOPECIA

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ABSTRACT

The androgenetic alopecia is a very common dermatological disorder affecting both men and women. In men, over age of 50 to more than 95% of them the hair loss is attributed to androgenetic Alopecia (AGA). In women AGA is less common with about 40% of women suffer from some degree of hair loss especially after menopause. Oral finasteride (FNS), a synthetic 4-aza-3-oxosteroid compound with low aqueous solubility. Blocks the peripheral conversion of testosterone to dihydrotestosterone (DHT). FDA has approved only minoxidil and finasteride for treatment of alopecia. Nanoparticles made up of biodegradable polymers have great potential for delivery of drug at target site, reduces the side effects and improve bioavailability of drugs. Solid lipid nanoparticles was prepared using lecithin/chitosan by solvent emulsification method followed by sonication. Finasteride loaded Lecithin Chitosan Nanoparticles (LCN) was characterized and optimized by parameters like particle size, zeta potential, surface morphology entrapment efficiency, in vitro release and stability studies. The optimized formulation was then further evaluated for the pharmacokinetic studies in Wistar rats. Finasteride -loaded LCN of particle size 245.5±7.60 nm, zeta potential 36±0.548 mV and entrapment efficiency 71.73±1.460%. showed optimum bioavailability in Wistar rats, maximum solubility and also showed good stability. This study confirm that the prepared using Lecithin Chitosan Nanoparticles (LCN) improve bioavailability and solubilty of the drug.

Keywords: Finasteride /pharmacokinetics. Finasteride /formulations. Finasteride /bioavailability. lecithin/chitosan. Lecithin Chitosan Nanoparticles (LCN).

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INTRODUCTION

In men over age of 50 were 95% of the hair loss is attributed to Androgenetic Alopecia (AGA). In women AGA is less common with about 40% of women suffer from some degree of hair loss especially after menopause. Male pattern baldness is known to depend on the presence of the androgen dihydrotestosterone (DHT) and on genetic predisposition. DHT is formed by testosterone through the action of the 5-α reductase enzyme. Though the disease is common but treatment is limited. Literature survey reveals that the nanoparticles are used as carrier for delivery of drug in the treatment of alopecia. Nanoparticles made up of different polymers had strong biological activity and large spectrum of action can be applied topically for the treatment of various types of alopecia.

FDA had approved only minoxidil and finasteride for treatment of alopecia. Literature survey reveals that finasteride have more efficacious for treatment of alopecia. Finasteride is a 5α-reductase inhibitor. It is a selective inhibitor of the type II and III isoforms of the enzyme. By inhibiting 5α-reductase, finasteride reduces the conversion of testosterone to dihydrotestosterone (DHT) in certain tissues in the body such as the prostate gland, skin, and hair follicles. FNS it is available in oral dosage form and had various side effects like gynecomastia and sexual dysfunction. Nanoparticles made up of biodegradable polymers have great potential for

delivery of drug at target site and reduces the side effects of drugs[1].

lecithin/ chitosan nanoparticles as an alternative colloidal carrier system to polymeric nanoparticles, solid lipid nanoparticles, liposomes and nanoemulsions. These nanoparticles were obtained from the supra-molecular self-organising interaction of negatively charged lipid material lecithin and of the positively charged polysaccharide.

The main objectives of studies are to evaluate of finasteride loaded lecithin chitosan nanoparticles in the treatment of alopecia. The purpose of study is the preparation and evaluation of FNS-loaded nanoparticles formula and incorporation of the same into gel for the treatment of androgenetic alopecia. In this study, nanoparticles loaded with FNS were produced by the modified method of spontaneous emulsification with diffusion of the solvent and characterized. The lecithin/chitosan(LCN) is one of the most used polymers mainly because of its biodegradability, biocompatibility capacity, and sustained drug release [2-4].

MATERIALS AND METHOD

The drug finasteride (FNS) was received as a gift sample from Sun Pharma Pvt. Ltd. Soya phosphatidylcholine (SPC) from Hi media Pvt Ltd and chitosan, ethanol, chloroform, methanol obtained from institutional store room all chemicals used are analytical grade.

Method of preparation of Lecithin Chitosan Nanoparticles (LCN)

Lecithin chitosan (LCN) nanoparticles were prepared by injecting ethanolic solutions of drug and lecithin in chitosan solution followed by continuous stirring. Negative charged lecithin and positive charged chitosan interact with each other and form Lecithin chitosan nanoparticles [4]. LCN were prepared according to the method reported by Lecithin (soya phosphatidylcholine) was dissolved in ethanol at a concentration of 2.5% (w/v). FNS was dissolved in the ethanolic solution of lecithin at different concentration [5]. Chitosan was solubilised in 0.27 N HCL at a concentration of 1% (w/v). FNS-loaded nanoparticle suspensions was obtained by injecting ethanolic lecithin/FNS solution (syringe inner diameter of 0.75mm) into water-diluted chitosan solutions that was mechanically stirred (1000 rpm). Appropriate volumes of 1% chitosan solution was diluted with distilled water to obtain L/C ratios of 40:1, 30:1, 20:1, 10:1 and 5:1 in the prepared nanoparticle suspensions. The blank nanoparticle (without drug) are also prepared by same procedure for comparison [5,6,7,8].

Figure 1: Method of preparation of FNS loaded LCN nanoparticles

Drug entrapment efficiency

Prepared nanoparticle suspension (600 µl) was centrifuged at 5000 rpm for 10 min in order to separate the possible precipitated in the preparation process [7]. 500µl of supernatant was transferred in to 5ml volumetric flask and disrupting solution (10% v/v acetic acid solution and 5 mL of ethanol 1:1) was added up to 5ml to form a solvent mixture sonicate for 30 min. centrifuged at 14000 rpm at 20°C for 1hr. supernatant was withdrawn and analysed for drug concentration by UV spectrophotometry [9]. Entrapment efficiency of the drug in SLNs was determined by quantifying the amount of free drug in the dispersion medium using below equation [10].

% EE= Theoretical Drug- Unentrapped Drug/ Theoretical Drug × 100

In-Vitro Drug Release Study

A Franz diffusion cell was used for In-vitro drug release study. Cellulose dialysis membrane was overnight kept in buffer solution for activation then membrane was placed horizontally in between donor and receptor compartment. The receptor compartments were filled with 20 ml of buffer mixture (4:1/buffer:ethanol), and it was maintained homogenous using magnetic stirrer. The temperature of receptor compartment was maintained at 37±0.5°C throughout the experiment. FNS formulation equivalent to 800 µg of FNS were applied onto the skin. Sample were collected at a predetermined time interval ½, 1, 2, 3, 4, 5, 6, 7, 8, and 24 hour, and replaced with the same amount of fresh medium. Absorbance of sample was taken using UV spectrometric method at 242 nm [11, 12, 13].

Particle Size and Zeta Potential

Particle size was done at Department of Pharmacy, Guru Ghasidas Vishwavidyalaya, Bilaspur C.G. measured by using Laser diffraction particle size analyzer (SALD 2201) and Zeta potential measurement was done at UIOP

Pt. Ravishankar Shukla University, Raipur C.G. measured by using zeta sizer (Malvern, Zetasizer nonseries). Nanoparticles suspension was diluted with distilled water to avoid multiple scattering. The measurements were performed at 25±0.5°C, at angle of 90° between the laser and detector.

Measurement of pH

Measurement of pH was done using digital pH meter (EI, Deluxe pH meter, model 101, India) [14].

Shape and surface morphology

Transmission electron microscope (TEM) of optimized formulation was done at all Indian Institute of Medical Science (AIIMS) Delhi using (TALOS, Transmission electron microscope). Staining of sample was done using 1% phosphotungustic acid. TEM image of optimized formulation and blank formulation was taken for comparison of formulation [15].

Drug Excipient Interaction Study by DSC and FT-IR spectroscopy

The differential scanning calorimeter (DSC) of finasteride, lecithin, chitosan, physical mixture of all the ingredient and formulation was performed to determine interaction between drug and excipients. The DSC thermograms of the drug and polymers were obtained using differential scanning calorimeter (TA instruments USA, DSC Q10 V9.4 Build 287 from the Department of Pharmacy, Birla Institute of Technology, Ranchi).

In which Infrared spectroscopy was performed using a Shimadzu FT-IR 8300 spectrophotometer and the spectra were recorded in duplicate for each of the samples at the region of 4000 to 400 cm^{-1}[16,17,18].

Biological Evaluation

Animals Male Wistar rats (12-16 weeks old, weighing 130-170 g) were used in the study. The animal were provided by institutional animal house of Department of Pharmacy, Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G.). Each rat of group II, III and IV were treated with testosterone solution (10 mg/ml) subcutaneously once a day for 21 days for inducing androgenetic alopecia [20] Each rat of group V, VI and VII were anesthetized by Ketamine i.p. Injection then hair of back of rat was removed by help of hair removal cream to induce diffusive alopecia. [21] Treatment in group II, III and IV was started after development of alopecia. Group I was treat with FNS formulation once a day, group III was treated with standard marketed formulation (5% minoxidil) once a day and group IV was treated with simple buffer with continuous s.c. injection of testosterone (10mg/ml) in all groups. Treatment in group V, VI and VII was started after removal of hair from the back of the rat's skin. Group V was treated with FNS formulation once a day, group VI was treated with VII standard marketed formulation (5% minoxidil)

RESULTS

Particle size and zeta potential

The particle size was observed in the range of 360 to 1129 nm with zeta potential -13 to -21.5 mV for the prepared batches of LCN. It has been observed that an increase in lipid content does not significantly affect the particle size; however, the particle size increased with increasing poloxamer 407 concentrations. Particle size of optimized nanoparticles was measured using particle size analyser (Malvern, zetasizer nonseries). Particle size of FNS loaded LCN nanoparticles was in range from 245.5 nm (F2) to 388.2 nm(F8). Particle size of the formulation was increased with concentration of chitosan. 245.5 nm (F8) particle size was taken as optimized formulation with desire particle size range depicted in figure-2.

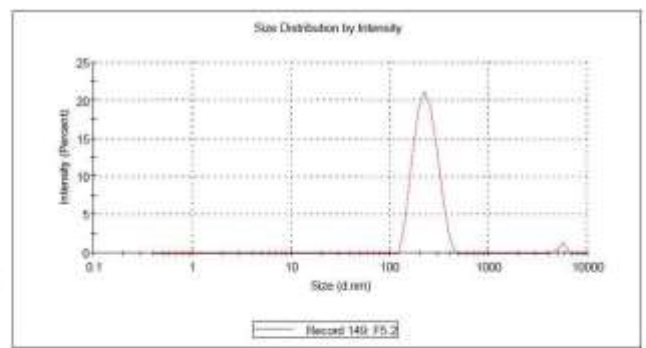


Figure-2: Particle size of optimized nanoparticle

Zeta potential of optimized nanoparticles was measured using Zeta sizer (Malvern, zetasizer nonseries). Zeta potential of nanoparticle was vary from negative [-0.157 mV(F8)] to positive [36.0 mV(F2)].

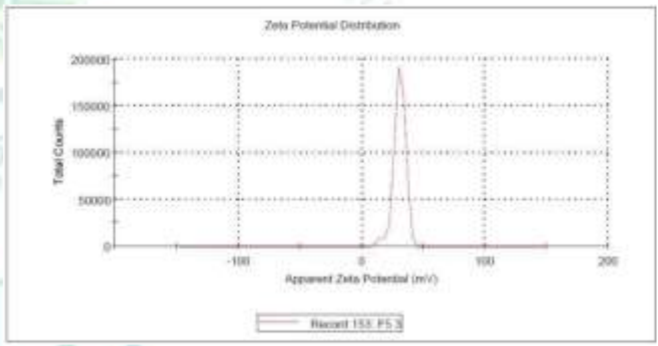


Figure-3: Zeta potential of optimized formulation

Drug entrapment efficiency

The percent entrapment was determined by UV spectrophotometry (λmax at 242 nm) method and observed to vary between 45.75% to 71.73% formulations batches F1 to F13. The percentage entrapment efficiency was observed to be decreased with the increase in concentration of polymer from 1.5% to 4% (i.e., formulations batches F1 to F13) and graph (figure-4). It has been observed that Finasteroid-loaded LCN F2 shown optimum percent entrapment 70.14% and F9 shown 87.87% is higher as compared to other formulations.

In-vitro drug release studies

In-vitro drug release study was performed by Franz diffusion cell and % cumulative drug was calculated. 63.178% of drug was released within 24 hrs. Graph shows almost linear release of drug. % drug release in different time interval and graph are deplicated in figure-5

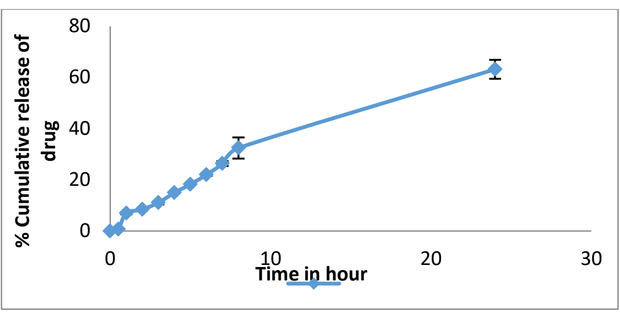
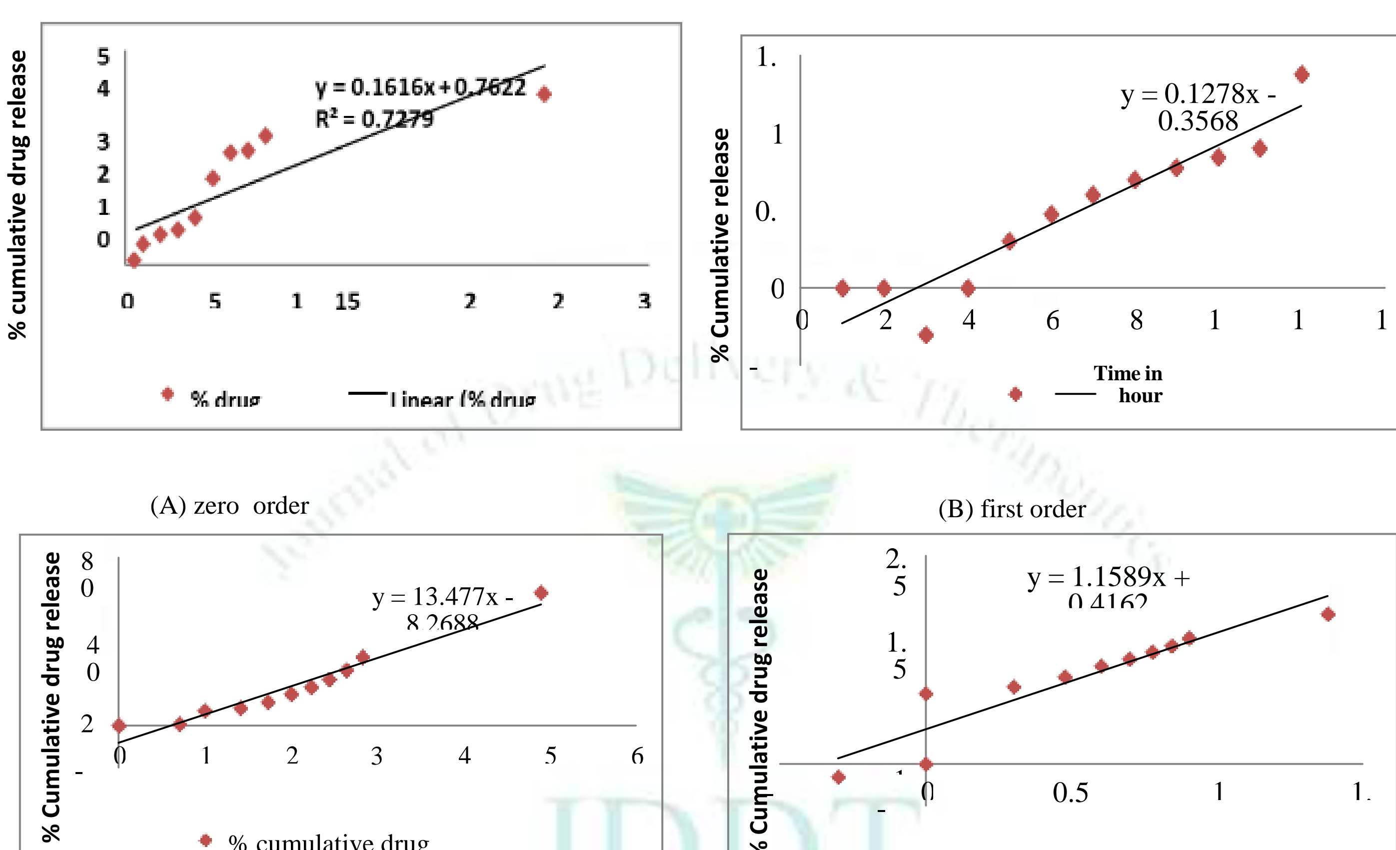


Figure-4: Graph of % cumulative of drug at different time

Release kinetics

The kinetics of drug release were evaluated to prove its design and performance. The mechanism and kinetics of drug release from LCNs were represented by plotting graphs of various kinetic models. In which rthe release profile of formulation F2 were obtained and to be fitted best with Korsmeyer-Peppas model. The release kinetic profiles of the optimized formulation (Fenasteroid-loaded SLN F2) such as zero-order, first-order, Higuchi and Korsmeyer-Peppas are shown in figure-5.



(A) zero order

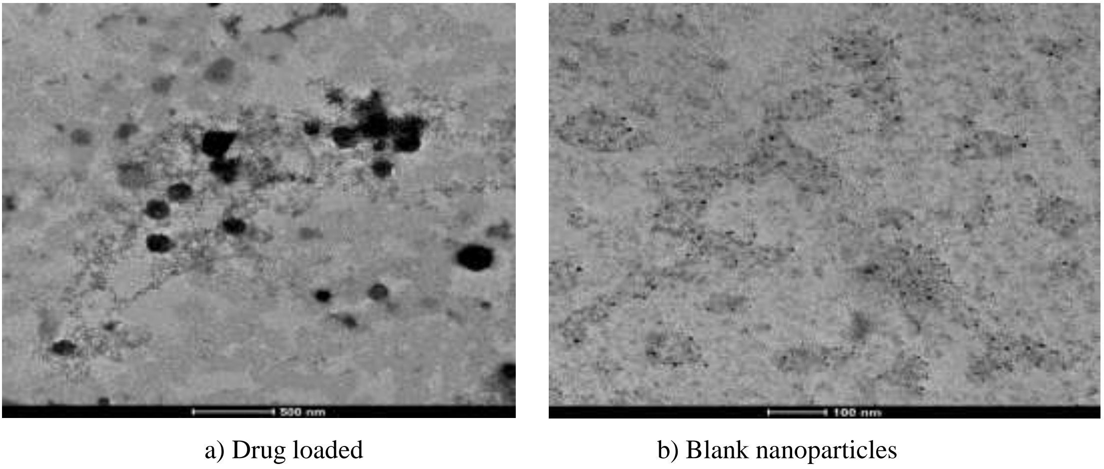
(B) first order

(C) Higuchi model

(D) KorsmeyerPeppas model

Figure-5: Release profile of Fenasteroid-loaded FNS F2, (A) zero order, (B) first order, (C) Higuchi model and (D) KorsmeyerPeppas model.

Measurement of Particle Shape (TEM)



a) Drug loaded

b) Blank nanoparticles

Figure-6: TEM image of a) Drug loaded and b) Blank nanoparticles

TEM images of nanoparticle confirm that particles are spherical in shape with narrow size distribution. TEM image of Fenasteroid (FNS) loaded nanoparticles was compared with blank nanoparticle. Blank nanoparticles shown particle size smaller then drug loaded nanoparticle.

The particle size and Zeta Potential of optimized nanoparticles was measured using particle size analyser (Malvern, zetasizer nonseries). Particle size of Fenasteroid (FNS) loaded Chitosan and Lecithin (LCN) nanoparticles was in range from 245.5 nm(F2) to 388.2 nm(F8). Particle size of the formulation was increased with concentration of chitosan. 245.5 nm (F8) particle size was taken as optimized formulation with desire particle size range

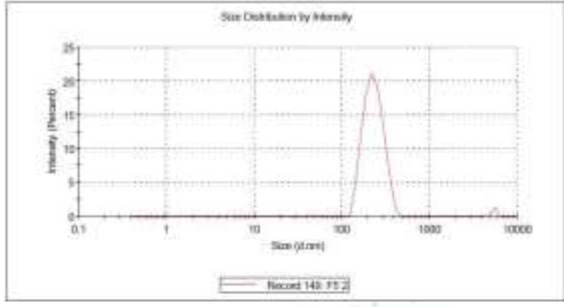


Figure-7: Particle size of optimized Drug loaded nanoparticle

Zeta potential of optimized nanoparticles was measured using Zeta sizer (Malvern, zetasizer nonseries). Zeta potential of nanoparticle was vary from negative [-0.157 mV(F8)] to positive [36.0 mV(F2)]. Zeta potential of particles was increase with decreasing the particle size so we found highest zeta potential in smallest particle that was optimized formulation (F8, 36.0nm) deplicate in table-7 and 8.

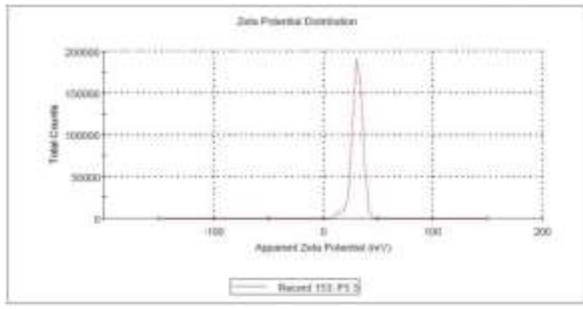


Figure-8: Zeta potential of optimized formulation

Drug Excipient Interaction Study by DSC

DSC thermogram of Fenasteroid(FNS), chitosan, soya lecithin and physical mixture all three components are shown in figure-12. Pure FNS showen a sharp peak at 258.12 °C corresponding to melting point of FNS that indicates its crystalline nature. Thermogram of physical mixture shown endothermic image of chitosan at 84.34°C and peak of lecithin at 194.99°C but does not show sharp melting peak of FNS peak shifted to 214.56°C near to peak of soya lecithin 212.19°C that indicates some interaction between excipients and drug or drug maybe dissolve in melted lecithin.

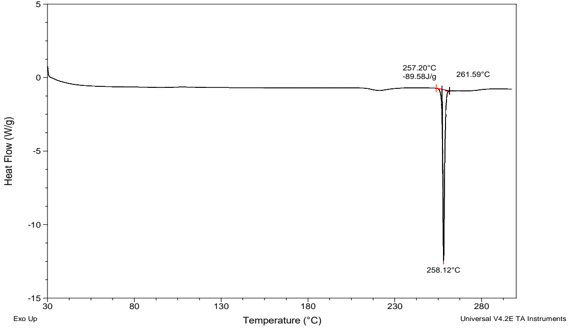


Figure-9: Thermogram of FNS

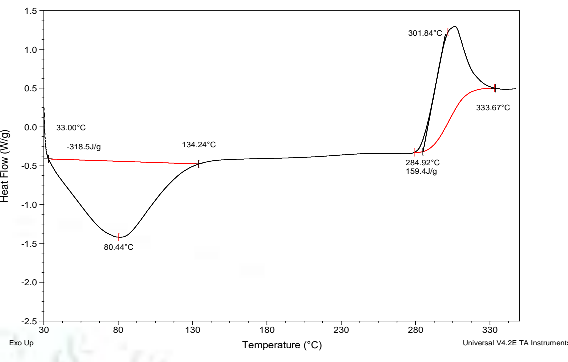


Figure-10: Thermogram of chitosan

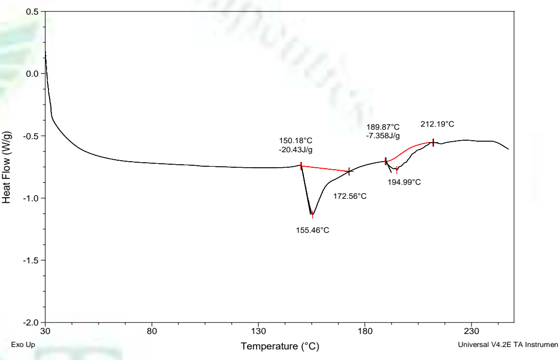


Figure-11: Thermogram of soya lecithin

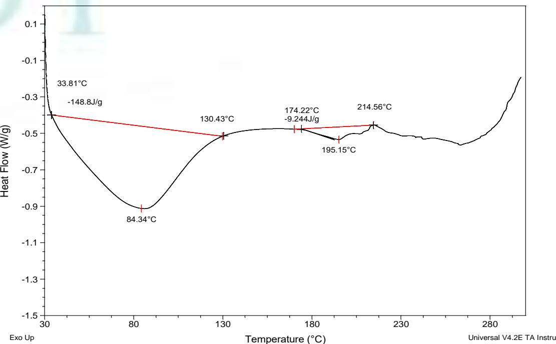


Figure-12: Thermogram of Physical Mixture

Drug Excipient Interaction Study by FT-IR

Interaction between drug and excipient was determined by Fourier Transform Infrared (FTIR) spectroscopy (BRUCKER ALPHA FTIR spectrometer). FTIR spectra of drug, excipients and formulation show all the characteristic peak of drug along with excipients all peaks was also show in formulation deplicated in figure-13,14,15 and 16. [19]

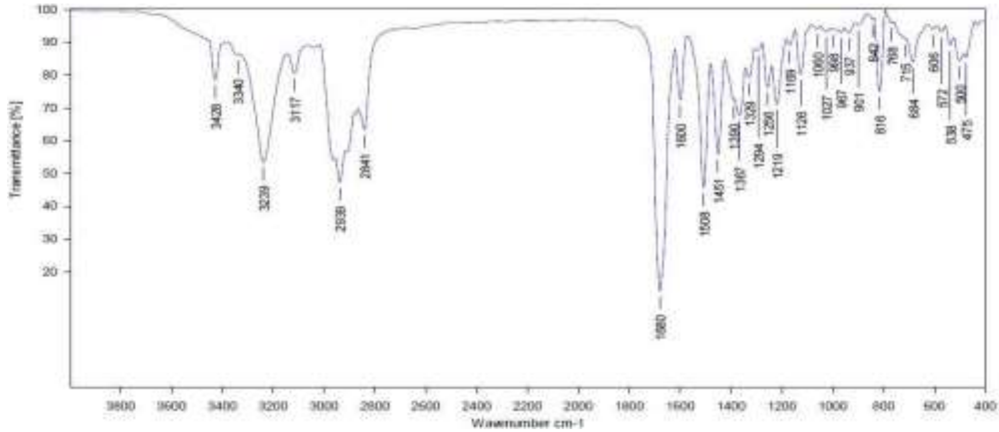


Figure-13: IR spectra of FNS

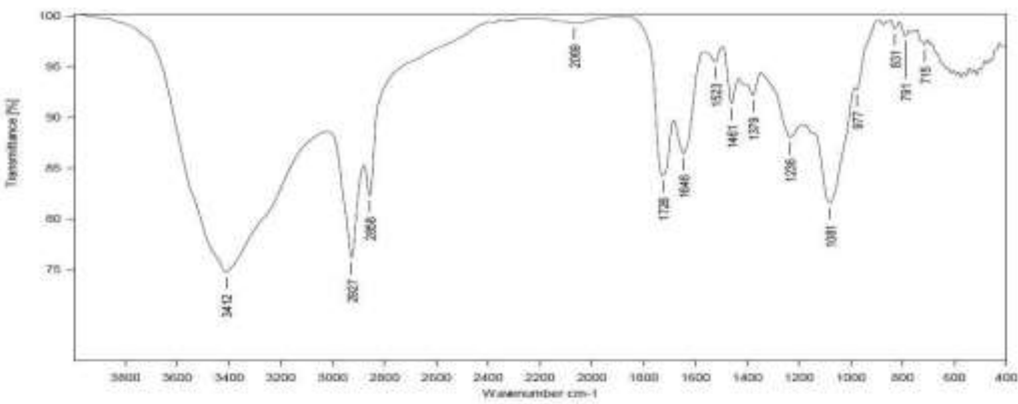


Figure-14: IR spectra of Formulation

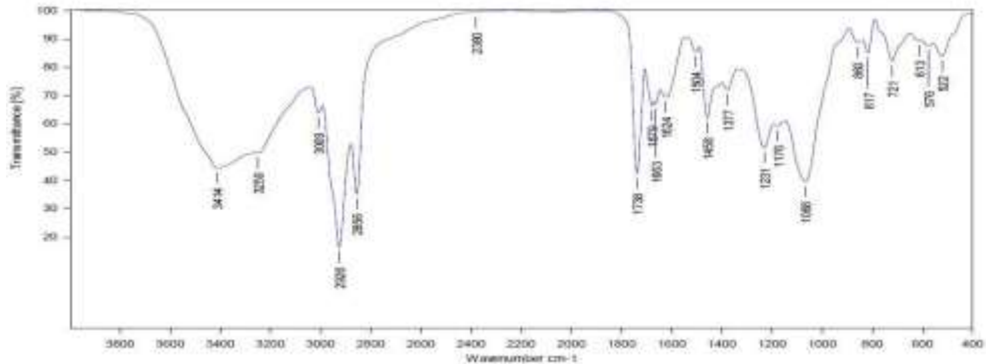


Figure-15: IR spectra of Physical mixture

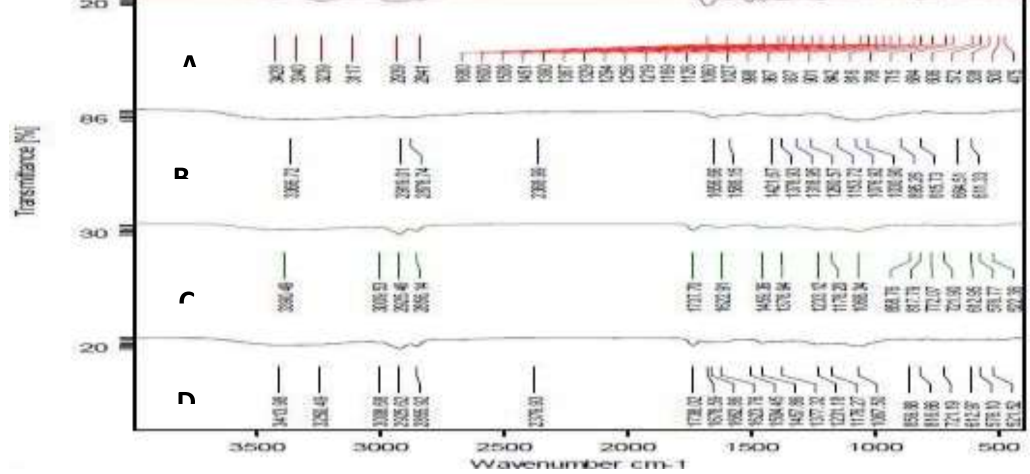


Figure- 16: Plot of IR spectra of (A) FNS, (B) Chitosan, (C) Lecithin, (D) Physical mixture

In vitro drug release study

Drug permeation via dorsal skin of rat was determined using franz diffusion cell. Result from study clearly indicates that nanoparticle formulation had limited drug flux across the skin. Data are shown in table-4 and graph is deplicated in figure-17.

Table-4: % Cumulative drug permeation through rat

S. No.	Time (hr)	% cumulative drug permeation
1	½	0.099±0.114
2	1	0.478±0.139
3	2	0.697±0.164
4	3	0.806±0.089
5	4	1.091±0.068
6	5	2.011±0.173
7	6	2.599±0.058
8	7	2.651±0.042
9	8	2.994±0.423
10	24	3.973±0.158

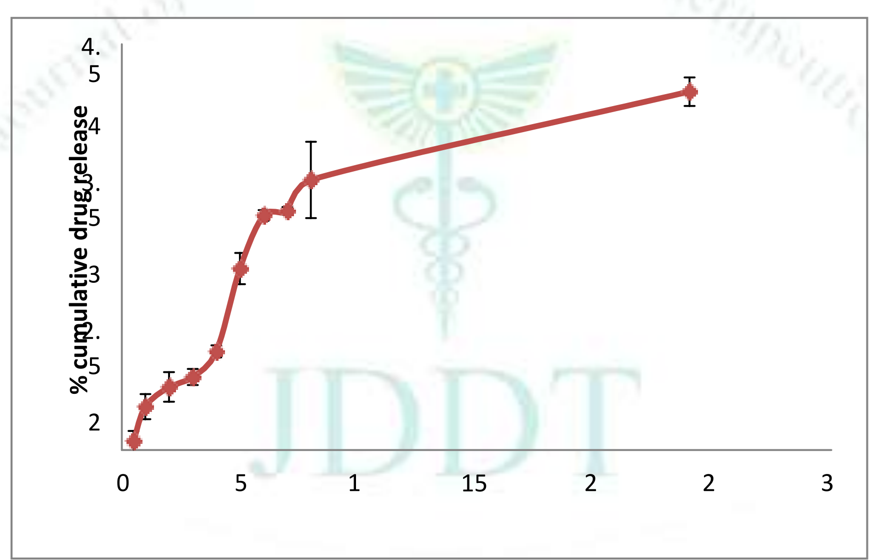


Figure-17: Graph of % cumulative drug release through rat skin

Evaluation of Alopecia

Model 1

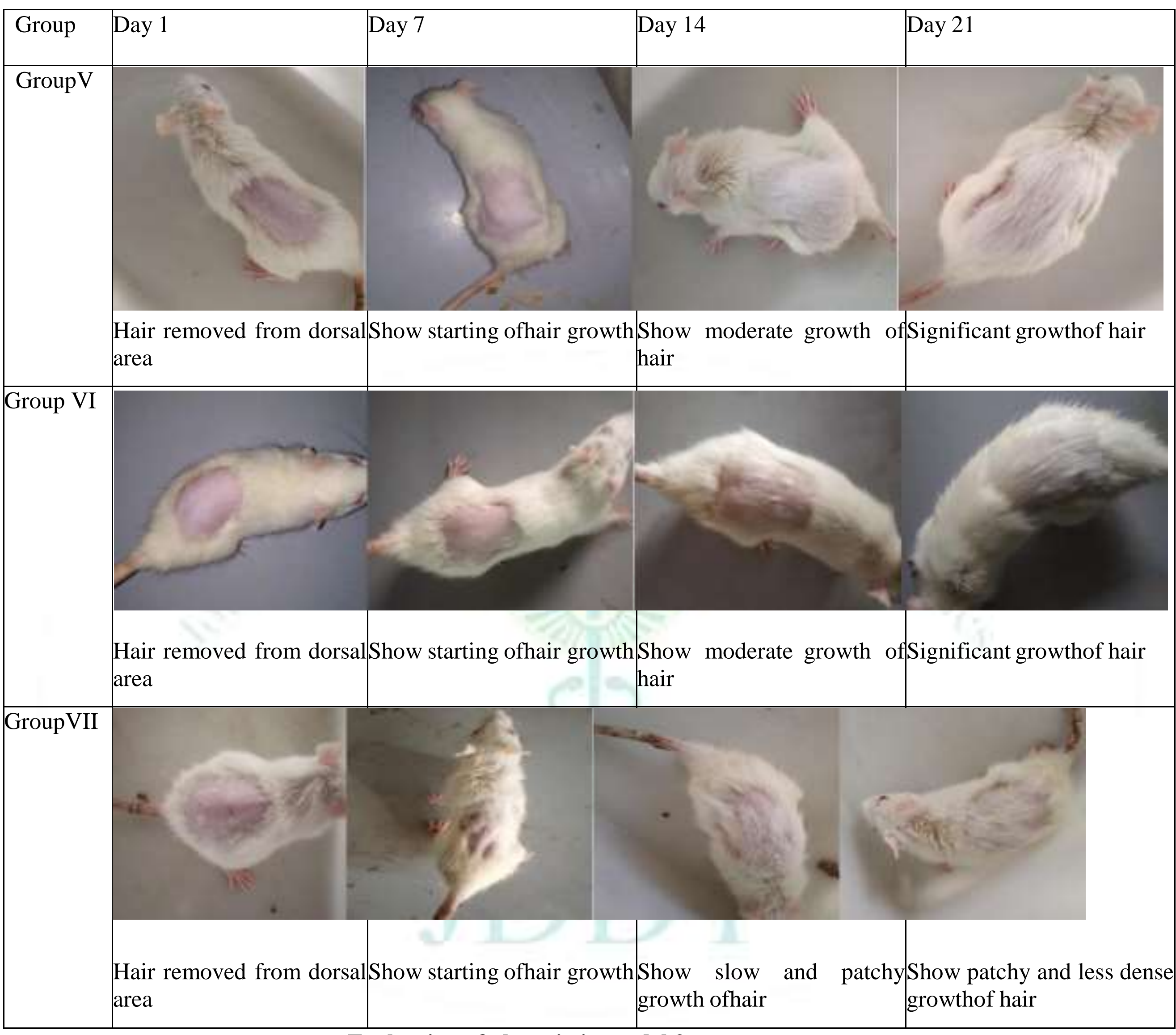
Animal treated with 0.1 ml testosterone s.c. injection (10mg/ml) for 21 days cause androgenetic alopecia on group II, III and IV. Testosterone was converted in dihydrotestosterone (DHT) in the presence of an enzyme 5-α reductases which accumulate in hair follicles and induce androgenetic alopecia. To prevent this conversion of testosterone start therapy with FNS formulation in group II simultaneously and observe further for reduction in alopecia and hair loss and regrowth of hair in 45 days . Group III treated with minoxidil also shown hair growth but group IV those treated with simple buffer show no significant improvement in hair growth as compare to group II and III.

Group	Day 1	Day 15	Day 30	Day 45
Group I	No treatment show normal hair	No treatment show normal hair	No treatment show normal hair	No treatment show normal hair
Group II	Show hair loss after testosterone treatment	Show reduction of hair growth	Reduction of hair growth and starting hair regrowth	Significant improvement on hair growth
Group III	Show hair loss after testosterone treatment	Show reduction of hair growth	Reduction of hair growth and starting hair regrowth	Significant improvement on hair growth
Group IV	Show hair loss after testosterone treatment	No reduction of hair loss	No reduction of hair loss	No reduction of hair loss

Evaluation of alopecia in model 1

Model 2

Animal treated with 0.1 ml testosterone s.c. injection (10mg/ml) for 21 days along with FNS formulation on group V, minoxidil on group VI and simple buffer on group VII simultaneously and observe further for reduction in alopecia and hair growth. Testosterone cause androgenic alopecia. Group V and VI those are treated with FNS formulation and minoxidil were reducing the alopecia i.e. those were able to prevent alopecia as compare to group VII which were treated with simple buffer.



Group	Day 1	Day 7	Day 14	Day 21
GroupV	Hair removed from dorsal area	Show starting ofhair growth	Show moderate growth of hair	Significant growthof hair
Group VI	Hair removed from dorsal area	Show starting ofhair growth	Show moderate growth of hair	Significant growthof hair
GroupVII	Hair removed from dorsal area	Show starting ofhair growth	Show slow and patchy growth ofhair	Show patchy and less dense growthof hair

Evaluation of alopecia in model 2

CONCLUSION

It can be concluded that the prepared solid lipid nanoparticles of finasteride (FNS) using Chitosan and Lecithin polymer enhanced bioavailability, drug release and solubility of the drug. Further, it can be said that LCN could be an alternative approach to other drug delivery systems and enhancing the bioavailability of the drugs and achieving nanoformulation with biodegradable polymers. Characterizations of the prepared formulations were done the basis of various parameters. It shown higher entrapment with lower particle size and have good zeta potential for improving their stability. In-vitro drug release study had shown linear first order release of drug from optimized nanoparticle formulation. In vitro skin permeation study was performed on rat skin that show limited drug flux across the skin. Two animal models are used for evaluation, in one model rats are treated with testosterone for 21 days for induction of alopecia. After 21 days treatment of rates with different formulation was start rats treated with FNS formulation shows good reduction of hair fall and hair regrowth as compare to other treatment. In other model hair of rats was removed by hair removal cream then treatment was continue for 21 days. Rats treated with FNS formulation show better regrowth of hair after 21 day as compare to group treated with minoxidil and without treatment growth. The in-vivo studies clearly show FNS loaded L/C nanoparticles are effective for reducing hair fall, improving hair regrowth means having marked potential for management and treatment of alopecia

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