Original Research Article

FORMULATION, EVALUATION AND STABILITY STUDIES OF DUTASTERIDE LOADED NIOSOMAL GEL

ABSTRACT

Introduction: Dutasteride, a synthetic 4-azasteroid belonging to the class of 5-α-reductase, a BCS class II drug, is used in treating Androgenetic Alopecia and Benign Prostatic Hyperplasia.
Aims: The study aims to formulate, evaluate and perform stability studies of Dutasteride-loaded topical niosomal gel.
Methodology: Formulation components such as, surfactants and cholesterol were tested for any drug/excipient interactions. Ether injection method was employed to prepare Niosomes. Five formulations were prepared and then assessed for their particle size, SEM, zeta potential, PDI, entrapment efficiency, drug content and in vitro diffusion studies. The optimized formulation F3 Consisted of Dutasteride, span 40 and cholesterol in the ratio 1:4:2. The optimized formulation (F3) was incorporated into a gel. Three gel formulations (FG1, FG2 and FG3) were prepared and were assessed for pH, viscosity, spreadability, drug content and in vitro diffusion studies. The optimized niosomal gel (FG2) consisted of 0.3% Optimized Niosomes and 0.75% Carbopol 934. The drug release kinetics studies were performed for the optimized gel. Finally, stability studies were performed at 30°C ± 2°C / 65% RH ± 5%.
Results: The optimized niosomes showed an Entrapment efficiency of 63.2%, zeta potential -22.7 mV, particle size 300.4 nm, drug content 86.23 % and 96.23% drug release in 8 hours. The optimized niosomal gel (FG2) consisted of 0.3% Optimized Niosomes and 0.75% Carbopol 934. The gel showed pH 4.7, viscosity 66213 cps, spreadability 15.7g.cm/sec, drug content 91.4% and percent drug release 91.45 % in 24 hours.The drug release kinetics studies showed that the optimized gel formulation (FG2) followed Higuchi model with R^2 value of 0.9975. The stability studies indicated that the optimized niosomal gel was stable for 90 days.
Conclusion: The stability studies confirmed the drug content and the physical nature of the gel for 90 days. Thus, the formulation can be regarded as stable and effective for drugs meant for topical application. Dutasteride gel formulations have the potential to enhance drug bioavailability by facilitating greater penetration of drugs with restricted permeability.

Keywords: Niosomal gel, Dutasteride, Niosomes, Androgenetic Alopecia, BCS class II

UNDER PEER REVIEW

1. INTRODUCTION

The classic male pattern baldness, or androgenetic alopecia, is a thinning of the hair that often appears in middle-aged men and gets worse with age. Interestingly, independent of hereditary susceptibility, testosterone hormone plays a major role in male androgenetic alopecia.

The enzyme 5α-reductase transforms testosterone in the hair follicle cells into dihydrotestosterone (DHT). There are two forms of it: type I and type II isoenzymes. The latter seems to have a special role in male pattern baldness; it is expressed in the prostate gland and other androgen-dependent tissues, including hair follicles. Specific androgen receptors are bound by DHT. The complex that is subsequently generated penetrates the hair cell's nucleus and interacts with DNA,

activating genes and causing the creation of proteins that lead to the slow transformation of normal hair follicles into "miniaturized follicles". So, this leads to shrinkage of hair follicles, shortening of the hair growth cycle, finally resulting in hair loss1,2.

The two medications that are most frequently used to treat alopecia are finasteride (1mg oral dose) and dutasteride (0.5mg oral dose). These drugs inhibit type I and II 5AR enzyme, preventing the conversion of testosterone into DHT. Dutasteride therapy reduces serum DHT (98%) significantly more than does finasteride (71%) and demonstrates significantly greater improvement in hair growth compared with finasteride3.

However, oral route treatment leads to significant reduction of DHT from the body and leads to serious, long lasting side effects related to fertility4. Topical delivery of drugs would help to tackle this problem. Therefore, there is a need for targeted delivery of the drug directly to the follicles, so that the drug remains localized in the follicles and will not reach the systemic circulation5,6,7,8.

Vesicular drug delivery systems are designed to enhance the delivery of drugs by encapsulating them within vesicles, which are small, spherical structures composed of lipid bilayers or other materials. These systems offer several advantages over traditional drug delivery methods, making them essential in modern pharmaceutical research and applications9. Advanced vesicular delivery systems like Transferosomes, ethosomes and transethosomes would enhance the delivery of drug into the systemic circulation.

Niosomes are vesicles composed of non-ionic surfactants which have demonstrated to significantly improve topical drug delivery and can also be employed in targeted drug delivery. Niosomes have been the subject of much research recently due to their potential as a vehicle for the administration of medications, antigens, hormones, and other bioactive substances. In addition, niosomes have been employed to address the issues of drug instability, insolubility, and fast degradation10,11,12,13,14. Based on various researches, Niosomes are able to deliver the drug to the deeper layers of skin, deep enough to reach the hair follicles and thus show enhanced therapeutic activity15,16,17,18,19. Therefore, a dutasteride loaded niosomal gel would be a promising solution to target the hair follicles and treat Alopecia.

So, the aim of this study was to incorporate the respective drug in this vesicular system and investigate the drug entrapment, drug release, drug-excipient interactions and stability studies.

2. MATERIAL AND METHODS

2.1 Materials

Dutasteride was obtained from Dr. Reddy's Laboratories Ltd., Hyderabad as a gift sample. Span 60, Span 40, Cholesterol, Carbopol, Methyl paraben were procured from s d fine-CHEM limited, Mumbai. Methanol, Potassium dihydrogen orthophosphate, Sodium hydroxide, Distilled water, Diethyl ether, Triethanolamine were procured from Thermo Fisher Scientific Pvt Ltd, Mumbai.

2.2 Methods

2.2.1 Calibration curve of pure drug Dutasteride:

Dutasteride solution of concentration of 100 ug/ml was analyzed at wavelength of 220-400 nm with UV-Visible Spectrophotometer. The stock solution (100 ug/ml) was then be used to prepare serial dilutions. Sub-samples of stock solution of dutasteride were pipetted out into a succession of 25 ml volumetric flasks and volume was made up to the mark with Phosphate buffer pH 7.4. to generate the concentration ranging from 5-30 µg/mL. The absorbance of each sample was determined at 240nm.

2.2.2 Drug Excipient interaction and Compatibility studies

FTIR studies were performed on the formulations to determine any drug/excipients interactions. Using the K-Br pellet method, FT-IR spectrophotometer was used to analyze the pure drug and the formulation. The analysis ranged from wave numbers 4000 to 400 cm-1 for the samples.

2.2.3 Formulation of Dutasteride loaded Niosomes:

Ether injection method was employed. Span and cholesterol were dissolved in (6 mL) diethyl ether, and Dutasteride 50mg was dissolved in (6 mL) methanol, then both the solutions were mixed together. 50 mL of phosphate buffer solution (pH

UNDER PEER REVIEW

7.4) was kept at 61-66°C and the previously prepared solution was administered at a rate of 1mL/min into this buffer solution by using a syringe and the solution was constantly stirred. After the solution was gradually introduced into the aqueous phase, temperature variations between the phases rapidly evaporated the ether, which led to immediate vesiculation and niosomes creation[20,21,22,23]. The formed niosomal suspension was sonicated for 2 min. The niosomal suspension was left overnight at 3-5°C before being refrigerated for future study.

Table 1: Formulation table of Dutasteride Niosomes

Formulation	Drug (mg)	Span 60 (mg)	Span 40 (mg)	Cholesterol (mg)	Drug/Surfactant/cholesterol ratio
F1	50	200	-	100	1:4:2
F2	50	100	-	100	1:2:2
F3	50	-	200	100	1:4:2
F4	50	-	100	100	1:2:2
F5	50	100	100	100	1:4:2

2.2.4 Characterization and Evaluation of Dutasteride Loaded Niosomes:

2.2.4.1 Vesicle Morphology:

The Form and structure of the drug loaded niosomal formulations was ascertained by Scanning Electron Microscopy (HITACHI)[20,21,24.]

2.2.4.2 Particle size, PDI and Zeta Potential:

Using Nano particle Analyzer (HORIBA SCIENTIFIC) to determine the average particle length, polydispersity index (PDI) and Zeta potential[20,22,23.]

2.2.4.3 Drug entrapment Efficiently:

The entrapment efficiency of niosomes was calculated with the usage of cooling centrifuge. The prepared formulations were put through the centrifugation at 17,000 rpm for at 4°C for 30 mins. After the supernatant was decanted, the amount of free drug was examined by UV/visible spectrophotometer at λmax of 240 nm[20,21,22]. The following formula was then used:

%Drug entrapment = (Total drug - Drug in supernatant / Total drug) x 100

2.2.4.4 Drug Content:

To determine the extent of dutasteride present in the niosomes, UV-visible spectrophotometer was used. In 10 mL methanol, Niosomes (100 mg) were dissolved by shaking for 5 mins. A mL from this solution was taken and made up to 10 mL with methanol. After that, sub samples were taken out, and absorbance was recorded at the appropriate wavelength[21,22,23.]

2.2.4.5 In Vitro diffusion Studies:

Franz-diffusion cell was used for this purpose. 10 ml of the prepared formulation was taken in the donor compartment and it was separated from the receptor compartment that was filled with phosphate buffer pH 7.4 by a membrane. A magnetic stirrer was used to agitate the medium at room temperature at 100 rpm. Samples were withdrawn and fresh buffer solution was added regularly at pre-decided time intervals. The collected samples after suitable dilution were analyzed spectrophotometrically at 240 nm[20,24.]

2.2.5 Formulation of niosomal gel of Dutasteride:

The Carbopol 934 concentrations (0.75%, 1%) were used to optimize the niosomal gel. Distilled water was used to disperse the polymer. The mixture was then continued to be stirred until it thickened. Following full dispersion, triethanolamine and methyl paraben were added. The polymer gel was combined with required quantity of the optimized niosomal dispersion while being constantly stirred. A sufficient amount of distilled water was added to get the required quantity of gel. A plain gel was also created for comparison study[25,26.]

Table 2: Formulation table for 0.3% Dutasteride in 10gm Niosomal Gel

Ingredients	Formulation 1 (Fg1)	Formulation 2 (Fg2)	Formulation 3 (Fg3)

UNDER PEER REVIEW

F3 optimized niosomes	-	0.3%	0.3%
Pure Dutasteride	0.3%	-	-
Carbopol 934	0.75%	0.75%	1%
Methyl Paraben	0.15%	0.15%	0.15%
Triethanolamine	q.s	q.s	q.s
Distilled Water	Up to 100ml	Up to 100ml	Up to 100ml

2.2.6 Characterization and Evaluation of Dutasteride Loaded Niosomal Gel

2.2.6.1 Physical Appearance:

The visual characteristics of every prepared gel formulation, including transparency, color, texture, stickiness, greasiness, smoothness, stiffness was determined[25,26.]

2.2.6.2 Homogeneity:

By visual checking, the uniformity of the Dutasteride Niosomal gels was verified[25,26.]

2.2.6.3 Viscosity study of Gels:

Niosomal gels were evaluated for their viscosity by Brookfield viscometer (DV-E, HA 22)[25,26.]

2.2.6.4 pH measurement:

A digital pH meter was used. Before proceeding, the pH meter was calibrated[26.]

2.2.6.5 Spreadability:

Good amount of sample was placed in between two glass slides and was squeezed to a consistent thickness by keeping one kg weight over it for 5 min[25]. The formula:
Spreadability = (Weight applied to the upper slide / Length moved on the glass) / Time

2.2.6.6 Drug content:

1 gm of the gel was combined with 100 ml of methanol. The stock solution was filtered and sub samples of different concentrations were made with appropriate dilutions and then analyzed at 240 nm. Drug content was determined[25,26.]

2.2.6.7 In vitro diffusion studies:

The permeation investigation was carried out in phosphate-buffered saline pH 7.4, that was sustained at 37 ± 0.2°C in a Franz diffusion cell (FDC). The stirring speed of the diffusion medium was kept at 100 ± 4 rpm. The membrane, which serves as a diffusion barrier between the donor and receptor compartments, was attached in the base of the FDC donor compartment. Formulation (10 ml) was kept in the donor compartment, and the study was continued for 8 h. The sample (1 ml) was taken out at pre-decided time intervals and the removed volume was replaced with fresh buffer. After passing through a 0.20-μm membrane filter, the extracted samples were examined[25,26.]

2.2.6.8 Drug Release kinetics studies:

Various release models like zero order, first order, Higuchi-equation, and Peppas-Korsemeyer were fitted to Niosomal gel formulation to ensure the drug release mechanism.

2.2.7 Stability studies:

In accordance with ICH requirements, the stability analysis of the Niosomal gels was conducted. Freshly made formulations were separated into groups and stored at 30°C ± 2°C / 65% RH ± 5%. for 90 days. During various time intervals, samples were taken out and various parameters were evaluated[26.]

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3. RESULTS AND DISCUSSION

3.1.1 Calibration Curve Results:

The calibration curve of the dutasteride was developed by dissolving in methanol for stock solution and made dilutions with phosphate buffer pH 7.4. The standard dutasteride graph demonstrated strong linearity, with $R^2 = 0.9989$, signifying its adherence to the “Beer-Lambert” law.

3.1.2 Drug and Excipient Compatibility Studies:

The functional groups in FT-IR spectrum of pure drug and formulation were almost similar and didn’t show any interactions and thus proves its compatibility.

3.1.3 Characterization and Evaluation Results of Dutasteride Niosomes

The Prepared Niosomes were characterized for various physicochemical properties.
Table 3 gives the results.

Table 3: Characterization and Evaluation Results of all Dutasteride Niosomal formulations

Formulation	Particle size (nm)	PDI	Zeta Potential (mV)	Entrapment efficiency (%)	Drug Content (%)
F1	290.3 ± 2.03	0.381	-20.5	59.8%	84.5
F2	140.8 ± 2.21	0.224	-19.1	53.6%	80.18%
F3	300.4 ± 2.38	0.302	-22.7	63.2%	86.23%
F4	152 ± 2.13	0.258	-15.6	51.1%	81.42%
F5	257.7 ± 2.23	0.318	-21.3	57.4%	83.28

3.1.3.1 Invitro diffusion studies:

Table 4: In vitro diffusion studies results of F1-F5 Niosomal formulations

Time (Hour)	F1 (% released)	F2 (% Released)	F3 (% Released)	F4 (% Released)	F5 (% Released)
0	0	0	0	0	0
1	34.14 ± 0.89	37.45 ± 0.18	45.14 ± 0.17	32.18 ± 0.20	40.15 ± 0.57
2	45.01 ± 0.17	44.18 ± 0.33	58.77 ± 0.23	43.01 ± 0.76	52.89 ± 0.54
3	52.33 ± 0.24	53.68 ± 0.85	71.52 ± 0.45	52.54 ± 0.13	64.19 ± 0.33
4	63.75 ± 0.41	58.82 ± 0.47	76.17 ± 0.68	58.21 ± 0.47	72.56 ± 0.75
5	72.41 ± 0.54	62.32 ± 0.31	79.28 ± 0.22	62.68 ± 0.51	76.84 ± 0.55
6	78.22 ± 1.22	67.59 ± 0.43	84.35 ± 0.13	66.48 ± 0.38	81.11 ± 0.24
7	83.08 ± 1.43	73.22 ± 0.93	92.11 ± 0.09	71.18 ± 0.18	84.80 ± 1.78
8	94.17 ± 1.69	85.85 ± 0.67	96.23 ± 0.34	78.50 ± 0.56	90.02 ± 1.47

*Average of three values
± Standard deviation

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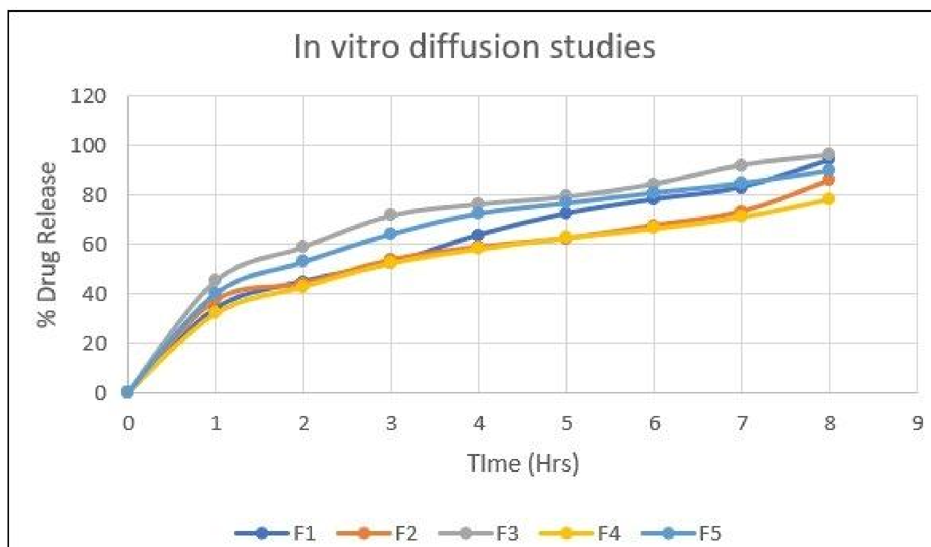

Fig.1 – In vitro diffusion studies of all niosomal formulations

Niosomes F3- formulation showed the appropriate particle size of 300.4nm, PDI of 302, zeta potential value of -22.7, % entrapment efficiency 63.2% and drug content was 86.23% and showed highest drug release across the membrane 96.23%. Hence it was selected as optimized formulation.

3.1.3.2 Scanning Electron Microscopy Evaluation and Zeta potential:

The optimized Niosomal formulation F3 was subjected to SEM analysis for describing the niosomes dimensions and form. Microscopic assessment showed, spherical Uni-lamellar vesicles size.

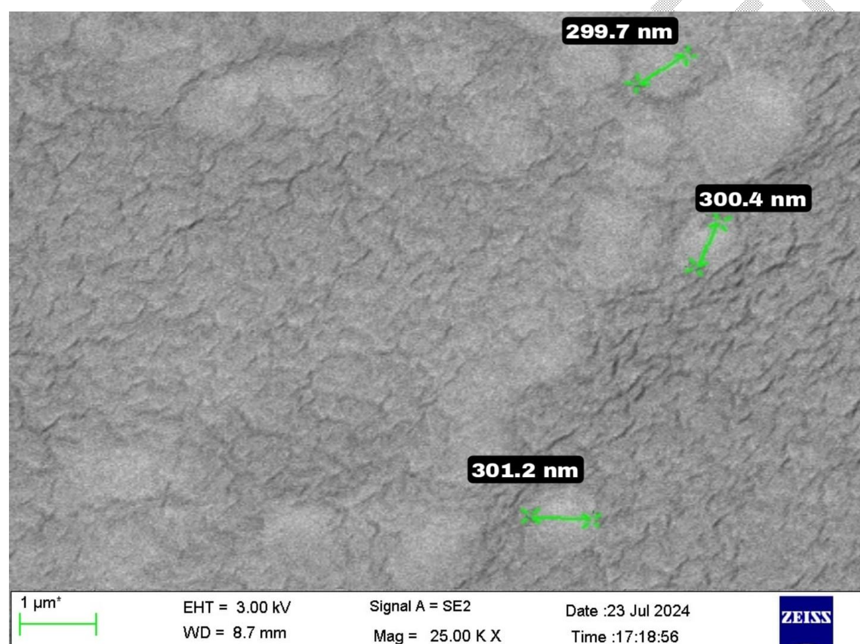

Fig. 2 – SEM Pic of F3 formulation

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Measurement Results

Date	:	12 July 2024 14:30:06
Measurement Type	:	Particle Size
Sample Name	:	S40 2-1 Q-SIZE
Scattering Angle	:	173
Temperature of the holder	:	25.0 deg. C
T% before meas.	:	81
Viscosity of the dispersion medium	:	0.895 mPa.s
Form Of Distribution	:	|Standard|
Representation of result	:	Scattering Light Intensity
Count rate	:	2568 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	226.7 nm	59.2 nm	206.6 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	226.7 nm	59.2 nm	206.6 nm

Histogram Operations

Size (Median)	:	217.9 nm
Mode	:	206.6 nm
% Cumulative (1)	:	5.0 (%) - 144.2 (nm)
% Cumulative (2)	:	10.0 (%) - 156.0 (nm)
% Cumulative (3)	:	20.0 (%) - 173.3 (nm)
% Cumulative (4)	:	30.0 (%) - 187.9 (nm)
% Cumulative (5)	:	40.0 (%) - 202.6 (nm)
% Cumulative (6)	:	50.0 (%) - 217.9 (nm)
% Cumulative (7)	:	60.0 (%) - 234.8 (nm)
% Cumulative (8)	:	70.0 (%) - 254.0 (nm)
% Cumulative (9)	:	80.0 (%) - 277.0 (nm)
% Cumulative (10)	:	90.0 (%) - 310.7 (nm)

Cumulant Operations

Z-Average	:	300.4 nm
PI	:	0.302

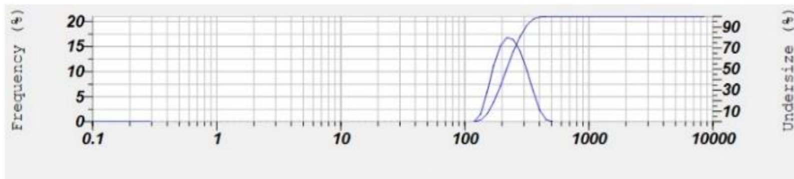

Fig. 3 – Particle size and PDI of F3 Formulation

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Measurement Results

Date	:	11 July 2024 17:02:23
Measurement Type	:	Zeta Potential
Sample Name	:	S40 2-1 Q-ZETA
Temperature of the holder	:	25.0 deg. C
Viscosity of the dispersion medium	:	0.894 mPa.s
Conductivity	:	0.425 mS/cm
Electrode Voltage	:	3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-2.2 mV	-0.000017 cm2/Vs
2	-41.8 mV	-0.000324 cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-22.7 mV
Electrophoretic Mobility mean	:	-0.000176 cm²/Vs

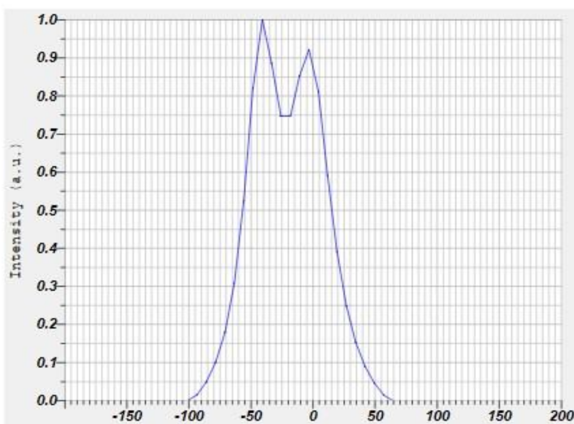

Fig. 4 – Zeta potential value of F3 Formulation

3.1.4 Characterization and Evaluation Results of The Prepared Dutasteride Niosomal Gels

Table 5: Evaluation Parameters of Gels

Formulation	pH	Viscosity (cp)	Homogeneity	Spreadability (g.cm/sec)	Drug content (%)
FG1 or Plain gel 0.75%	4.3	67175	Good	15.9	85.5
FG2 or 0.75% optimized gel	4.7	66213	Good	15.7	91.4
FG3 or 1% gel	5.2	65987	Good	15.57	88.6

3.1.4.1 Invitro diffusion Study:

Table 6: In vitro diffusion studies of niosomal gels

Time (hrs)	FG1 (% released)	FG2 (% released)	FG3 (% released)
0	0	0	0
1	3.87	16.76	9.21
2	7.38	24.38	14.38
4	10.23	33.74	20.74
6	15.28	42.23	28.83
8	20.43	50.28	35.28
24	57.55	91.45	82.45

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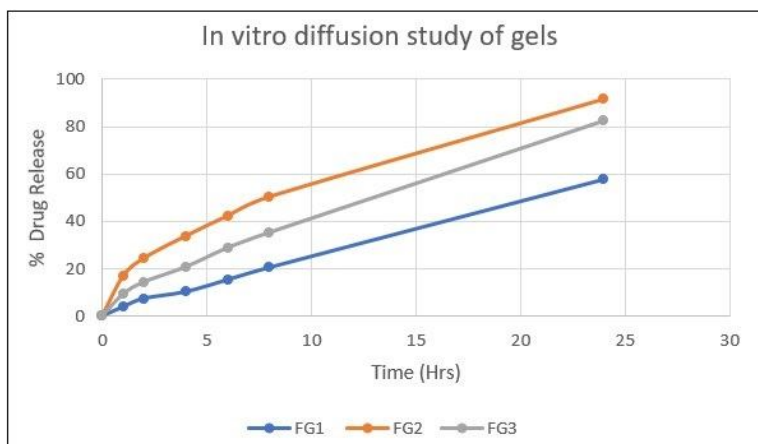

Fig. 5 – In vitro diffusion studies of all niosomal gels

FG2 gel formulation showed maximum drug release (91.45 %), good homogeneity, maximum drug content, good viscosity and pH. As a result, it was regarded as the optimal gel formulation.

3.1.4.2 Drug Release Kinetic Studies:

Table 7: Regression (R^2) values of various pharmacokinetic profiles of the optimized gel formulation (FG2)

Zero order	First order	Korsmeyer-Peppas	Higuchi
0.922	0.9882	0.9921	0.9975

The drug release kinetics and Release mechanism were applied to the prepared 0.75% Carbopol Niosomal gel (FG2) or the optimized gel. The drug release time profile was fitted to the formulations using a variety of equations, including Zero order, First order, Korsmeyer-Papas and Higuchi. The drug release kinetics showed that the gel followed Higuchi model (R^2 = 0.9975).

3.1.5 Stability Studies

Table 8: Stability studies of the optimized gel (FG2)

Formulation	FG2			
Storage Condition	30°C ± 2°C / 65% RH ± 5%			
Time Intervals (Days)	0	30	60	90
pH	4.7	4.6	4.6	4.5
Viscosity (cP)	66213	65734	64345	63515
Spreadability	15.7	15.4	15.3	15.2
Drug Content Uniformity (%)	91.4	91.2	91.1	90.9

The stability study of the niosomal gel was performed as per ICH guidelines. The gel's physical form, pH, viscosity, homogeneity and drug content was good during the period of storage. This shows the stability of the gel formulation.

4. CONCLUSION

The current study's objective was to prepare, characterize and evaluate niosomal gel loaded with Dutasteride. According to pre-formulation research studies, Dutasteride was soluble in Methanol. Furthermore, FTIR analysis showed that the drug and the excipients showed no interactions. Among the 5 formulations of niosomes prepared, the F3 formulation prepared with the drug, span 40 and cholesterol in 1:4:2 ratio was the optimized formulation, with an In vitro release of approximately 96.23% within 8 hours, 86.23% drug content, entrapment efficiency of 63.2%, -22mV zeta potential, and a particle size of 300.4 nm. SEM analysis of optimized dutasteride niosomes revealed spherical, unilamellar vesicles. The optimized niosomes (F3) were incorporated into three gel formulations. Consequently, the optimized niosomal gel (FG2) had a pH of 4.7, viscosity 66213 cps, spreadability 15.7 g.cm/sec, 91.4% drug content and 91.45% drug release in 24

UNDER PEER REVIEW

hours. The drug release kinetics showed that the gel followed Higuchi model. The stability studies confirmed the drug content and the physical nature of the gel for 90 days. Thus, the formulation can be regarded as stable and effective for drugs meant for topical application. Dutasteride gel formulations have the potential to enhance drug bioavailability by facilitating greater penetration of drugs with restricted permeability.

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