WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 5, 656-674. Research Article ISSN 2277– 7105

FORMULATION DEVELOPMENT AND EVALUATION OF ANTIFUNGAL PRONIOSOMAL GEL FOR TOPICAL APPLICATION

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Article Received on
05 March 2017,

Revised on 22 March 2017,
Accepted on 13 April 2017

DOI: 10.20959/wjpr20175-8300

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ABSTRACT

The purpose of present research work was to formulate the antifungal Proniosomal gel of Terbinafine hydrochloride with the aim to prolonged drug action and also to overcome the demerits of liposomes and niosomes. In this research work Proniosomal gel was formulated by using Terbinafine hydrochloride as antifungal drug, span 40 and lecithin as a penetration enhancer, cholesterol as a stabilizer and ethanol solvent as solubilizer. Total nine formulations of Proniosomal gel were prepared. The formulated Proniosomal gel was evaluated for the different parameters such as physical characterization, pH, spreadability, viscosity, entrapment efficiency, In-vitro drug release, vesicles size and shape and stability. The formulation F5 out of nine formulations was selected as optimized formulation, which showed the highest % entrapment efficiency (90.54±1.3%), prolonged drug release (66.6±0.9) in 24 hrs. The vesicles formed were in spherical shape identified by optical microscopy. The optimized formulation (F5) was stable for one month of time period. FTIR results showed that there was no interaction between the drug and excipients. Proniosomal gel can be used for the prolonged and localized action of drugs.

KEYWORDS: colloidal drug delivery systems, niosomes, liposomes, antifungal drug, topical drug delivery systems.

INTRODUCTION

Drug delivery system using colloidal particulate carriers such as liposomes and niosomes have distinct advantages over conventional dosage forms. i.e. shows strong potential for effective drug delivery to the site of action, biologically inert in nature, release the drug in sustained and controlled manner and also have ability to entrap both hydrophilic and

lipophilic drugs. However, remain significant problems like physical instability in general application of liposomes and niosomes for drug delivery. Provesicular concept has evolved to resolve the stability issues pertaining to conventional vesicular systems. Proniosomes minimizes problems associated with niosomes i.e. physical instability such as aggregation, fusion and leakage. It also provides additional convenience in transportation, storage and dosing.[1,2] Proniosomal gel offers a great potential to reduce the side effect of drug and increase the therapeutic effectiveness of topical drug delivery.

Proniosomal gel is a semisolid liquid crystalline product of nonionic surfactant easily prepared by dissolving the surfactant in a minimal amount of an acceptable solvent and small amount of aqueous phase. The Proniosomal gel is becoming more popular due to ease of application and better percutaneous absorption than other semisolid preparation.[3]

Gel can resist the physiological stress caused by skin flexion, mucociliary movement, adapting of shape of the applied area and for controlling drug release.

The purpose of topical and dermatological dosage form development is to conveniently deliver drug molecule at a localized area of the skin.[4]

Fungal infection of the skin can be treated by antifungal drugs. Terbinafine hydrochloride is a synthetic allylamine antifungal which is fungicidal against dermatophytes, molds, fungi and some yeast. It is the agent of choice for treatment of dermatophyte nail infection.[5] It acts as a competitive inhibitor of "squalene epoxidase" An early step enzyme in ergosterol biosynthesis by fungi. Accumulation of squalene within fungal cell appears to be responsible for the fungicidal action. Side effects of oral terbinafine are gastric upset, rashes, taste disturbance.[6]

In topical administration of antifungal, the drug substances should pass the stratum corneum, which is the outer most layer of skin, to reach lower layer of the skin, particularly into viable epidermis. In this context, the formulation may play a major role for penetration of drug into skin. Development of alternative approaches for topical treatment of fungal infection of skin encompasses new carriers systems for approved and investigational compound. Delivery of antifungal compound into the skin can be enhanced with the carriers including colloidal systems, vesicular carriers and nanoparticles.[7,8]

The objective of the study was to optimize and evaluate various antifungal proniosomal gel

formulations. The antifungal proniosomal gel formulations were prepared by using Terbinafine hydrochloride as antifungal drug, nonionic surfactant (span 40), different amount of cholesterol and soyalecthine. We also investigated the stability and feasibility of Proniosomal gel of Terbinafine hydrochloride for topical application.

MATERIALS AND METHODS

The Material required in formulation development and evaluation of proniosomal gel are listed in table given below.

Table No. 1: List of Materials

S.No.	Materials	Sources
1	Drug (Tebinafine)	Sri Ram Healthcare (P) Ltd, Baddi
2	Span 40	Central drug house (P) ltd
3	Cholesterol	Merck Pvt ltd
4	Soya lecithin	Central drug house (P) ltd
5	Ethanol	Merck Pvt Ltd
6	Potassium dihydrogen phosphate	Merck Pvt Ltd
7	Sodium hydroxide	Merck Pvt Ltd

The drug Terbinafine hydrochloride was procured as gift sample from Sri Ram Healthcare (P) Ltd, Baddi (Himanchal Pradesh) and other excipients were provided by the department of pharmacy M.J.P. Rohilkhand university, Bareilly (Uttar Pradesh).

Preformulation Studies

Preformulation studies are the first step in the rational development of dosages forms. It can be defined as an investigation of physical and chemical property of a drug substance alone and in combined with excipients.

Preformulation studies not only helps to guide dosages form selection, but also provide knowledge that how drug products should be processed and stored to ensure their quality.[9,10]

Organoleptic Properties of Drug

The drug sample (Terbinafine hydrochloride) was noted for its organoleopic properties such as colour, odour, taste and appearance.[5]

Melting Point

Melting point of the drug sample (Terbinafine hydrochloride) was determined using capillary tube method. Small amount of powdered drug was filled inside the thin capillary tube and

sealed from one side by melting. The capillary was placed into the melting point apparatus. Thermometer was also placed in the apparatus. After some time at specific temperature drugs were melted that was the melting point of drug. The melting point range of the Tebinafine hydrochloride is 195- 197ºC.[11]

Partition Coefficient of Drug

For determination of partition coefficient of drug, equal ratio of chloroform and water, 10ml of each was taken. In this mixture excess amount of drug was added and shake properly in separating funnel for mixing the drug with both phases and leave the mixture of solution for 24 hr for proper separation into two phases such as chloroform and water. After 24 hrs chloroform and water phases was separated individually in beakers. Sonicate the obtained filtrate for better clearance of the solution for 15 minute at 80 Hz. Perform the dilution and check the absorbance at 283 nm. Repeat the same procedure in triplicate for better accuracy.[12]

Solubility of Drug

About 5mg Terbinafine hydrochloride was added to 10 ml of various solvent and sonicated for 10 minutes and inspected visually for solubility and compared with standard.[13]

Table No. 2: Solubility of Terbinafine Hydrochloride

S.No.	Solvent	Solubility
1	Water	Slightly soluble
2	Methanol	freely Soluble
3	Ethanol	Freely Soluble
4	Acetone	Slightly Soluble

Determination of Wave Length (λmax)

Wave length maxima (λmax) of terbinafine hydrochloride was determine as follows.

Preparation of standard stock solution

For the preparation of standard stock solution, 10mg of Terbinafine hydrochloride was weighed and transferred to 100ml volumetric flask. It was dissolved in small amount of ethanol: water (40:60) and then volume was adjusted to up to the mark to make final concentration of 100µg/ml.

Selection of analytical wavelength (λmax)

From stock solution withdrawn 0.5 ml and 4.0 ml solution and volume make up to 10ml with ethanol: water (40: 60). The resulting obtained concentration of solution 5µg/ml and 40µg/ml. Absorbance was determined by UV spectrophotometer, the maximum absorbance showed by UV spectrophotometer at λ max in range of 200-400 nm.

Preparation of dilution samples

Dilution samples were prepared from stock solutions (100µg/ml). The 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 ml solution was withdrawn from stock solution and transferred to 10 ml volumetric flask then volume make up to 10ml with ethanol : water (40:60) to got serial dilution 5, 10, 15, 20, 25, 30, 35, 40 µg/ml.

Preparation of calibration curve of Terbinafine hydrochloride

From the above prepared dilutions (5, 10, 15, 20, 25, 30, 35 and 40 µg/ml), the spectra were recorded on wavelength at 283 nm and calibration curve was plotted.[14,15]

FTIR studies of Drug and Excipients

FTIR spectra was recorded for Terbinafine hydrochloride and prepared proniosomal gel using Thermoscientific FTIR in the region of 4000-500 cm^{-1}. Proniosomal gel was diluted with the phosphate buffer (7.4) to determine its FTIR spectra. Then the characteristics peaks of specific functional of drug were matched with the standard.[16]

Formulation development of Proniosomal gel

The proniosomal gel was formulated by using co-acervation phase separation method.

Coacervation phase separation method - In this method the required amount of span 40, soya lecithin, cholesterol and drug (Terbinafine hydrochloride) are taken in a clean and dry wide mouthed glass vial and alcohol was added to it. After warming, all the ingredients were mixed well with a glass rod and the open end of the glass vial was covered with a glass lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture is dissolved completely. Then the aqueous phase was added and warmed on a water bath till a clear solution was formed which was then converted into proniosomal gel on cooling The gel so obtained was preserved in the same glass bottle in dark conditions for its characterization.[1,17,18]

Formulation Chart

The antifungal proniosomal gel was prepared by Coacervation phase separation method. For the formulation of proniosomal gel, Drug (Terbinafine Hydrochloride) and Excipients (span 40, cholesterol, soyalecthine and ethanol) were taken as amount given in the formulation chart given below.

Table No. 3: Formulation Chart

F. C	Drug (mg)	Span40 (mg)	Cholesterol (mg)	Soya lecithin (mg)	Ethanol (ml)
F1	100	900	200	900	1.5
F2	100	1350	200	900	1.5
F3	100	1800	200	900	1.5
F4	100	900	250	900	1.5
F5	100	1350	250	900	1.5
F6	100	1800	250	900	1.5
F7	100	900	300	900	1.5
F8	100	1350	300	900	1.5
F9	100	1800	300	900	1.5

Evaluation parameters for Proniosomal gel

The prepared Proniosomal gel was evaluated for the following parameters as.[19,20,21]

Physical evaluation

The prepared gel was evaluated visually for colour, consistency, homogeneity and phase separation.

Viscosity determination

The measurement of viscosity of prepared proniosomal gels was done by using Brookfield viscometer. Proniosomal gel was taken in beaker and placed below the T- shaped spindle (spindle no. - 92) then rpm was set set at 2. Then on the motor of the viscometer and note down the readings.

Percent Entrapment Efficiency

The percent entrapment efficiency was calculated by taking the 50 mg of formulated proniosomal gel in a glass tube and 10 ml of phosphate buffer (ph 7.4) added to this glass tube. Then this aqueous suspension was sonicated by sonicator, followed by centrifugation at 900 rpm at 20ºc for 30 min. The supernatant solution was separate out from this aqueous suspension. Then withdrawn 0.1 ml from supernatant solution and transferred to 10 ml

volumetric flask then volume is make up to 10 ml. The solution was filtered and assayed by UV spectrophotometer for un- entrapped terbinafine hydrochloride content at λmax 283 nm. The percentage of the drug entrapment efficiency (% E.E.) was calculated by following equation.

$$\text{Percent Entrapment efficiency} = \frac{\text{Total amount of dug} - \text{Un Entapped drug}}{\text{Total amount of drug}} \times 100$$

In- vitro drug release study

In vitro release studies of Terbinafine hydrochloride from proniosomal gel were carried out by drug diffusion studies through cellophane membrane. For the determination of cumulative % drug release, firstly tied a boiled cellophane membrane on the one end of double cut tube then Proniosomal gel preparation (50 mg) that was diluted with 10ml phosphate buffer (ph 7.4) taken in a double cut tube and suitably suspended this tube in a beaker containing 100ml of diffusion medium (phosphate buffer saline 7.4 ph). The medium was maintained at room temp. It was stirred by means of magnetic stirrer at a constant speed. Sample of 2ml (diffusion medium) was withdrawn at a specific interval and replaced with the fresh diffusion medium, to maintain the sink condition. The samples were measured spectrophotometrically at 283nm. Then absorbance was note down. The cumulative % drug release of drug from proniosomal gel was calculated by putting the absorbance values in the calibration curve equation.

Vesicle shape

Proniosomal gel gives niosomes vesicles on hydration. The vesicular structure was observed for diluted formulation of proniosomal gel with phosphate buffer (ph 7.4) under the electronic optical microscope at 100x magnification.

Stability studies

To determine the stability of the formulated Proniosomal gels, the optimized formulation was stored in airtight sealed vials at various temperature conditions like refrigeration (2ºC - 8ºC) and room temperature (25ºC ± 0.5ºC). Surface characteristics, colour and percentage entrapment efficiency of niosomes derived from proniosomal gel were selected as parameter for evaluation of the stability, since instability of the formulation would reflect in drug leakage and a decreased in percentage drug entrapment efficiency. The proniosomal gel after a time period of one month, observed for change in colour, shape of vesicles and entrapment efficiency.

RESULS AND DISCUSSION

Preformulation Studies of Drug (Terbinafine hydrochloride)

Preformulation studies of drug (Tebinafine hydrochloride) was carried on the basis of following parameters

Identification and characterization of Terbinafine hydrochloride

The results of the various parameters of identification and characterization of Terbinafine hydrochloride as discussed below.

Organoleptic Properties of Drug

The drug was identified on the basis of organoleptic properties.

Table No. 4: Organoleptic Properties of Terbinafine Hydrochloride

Colour	Offwhite
Odour	Odourless
Taste	Bitter
Appearance	Fluffy powder

Melting point of drug

The melting point of Tebinafine hydrochloride was found to be 197ºC. The normal range of the melting point of Tebinafine hydrochloride is 195-197ºC, shows that melting point of drug was lying between melting point range. Melting point indicates the purity of drug.

Partition coefficient of drug

Partition coefficient of the Terbinafine hydrochloride was found to be 5.39 (log $P_{O/W}$) and the normal range is 5.53 (log $P_{o/w}$).

Solubility of drug

Terbinafine hydrochloride was freely soluble in anhydrous ethanol and methanol, slightly soluble in acetone and very slightly soluble in water that shows it is lipophilic in nature.

Determination of λmax of Terbinafine hydrochloride

The λmax of the Terbinafine hydrochloride was found to be 283nm in ethanol: water (40:60). The spectra of Terbinafine hydrochloride by U.V. spectrophotometer is shown below.

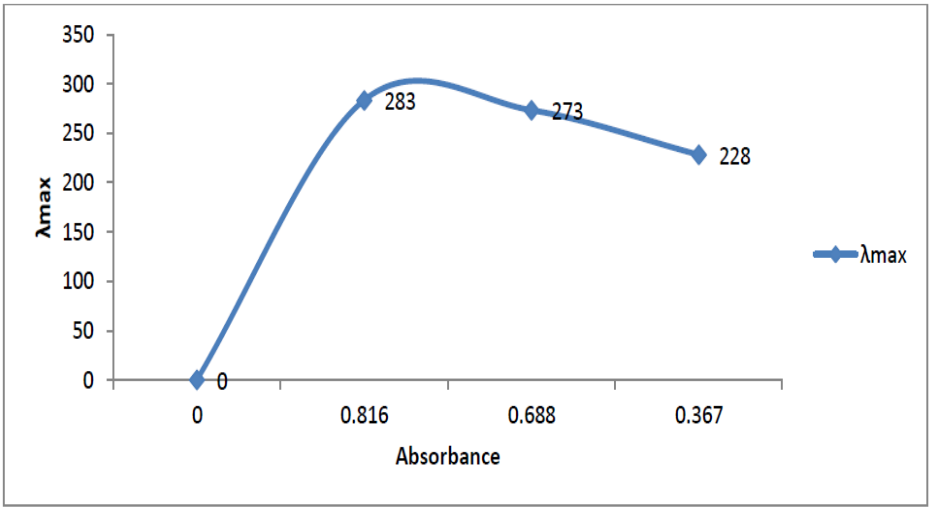

Fig. No. 1: Spectra of Terbinafine Hydrochloride in ethanol: water (40:60).

Calibration curve of Terbinafine hydrochloride

For the preparation of calibration curve, samples was prepared from stock solution (5, 10, 15, 20, 25, 30, 35, 40 ug/ml). The absorbance of sample was taken at 283 nm.

Table No. 5: Analytical data for calibration curve of Terbinafine Hydrochloride

S.No.	Concentration(µg/ml) in ethanol: water(40:60)	Absorbance
1	**0**	**0**
1	5	0.132
2	10	0.212
3	15	0.321
4	20	0.418
5	25	0.521
6	30	0.617
7	35	0.716
8	40	0.812

The graph plotted between concentration and absorbance was found to be linear and straight line.

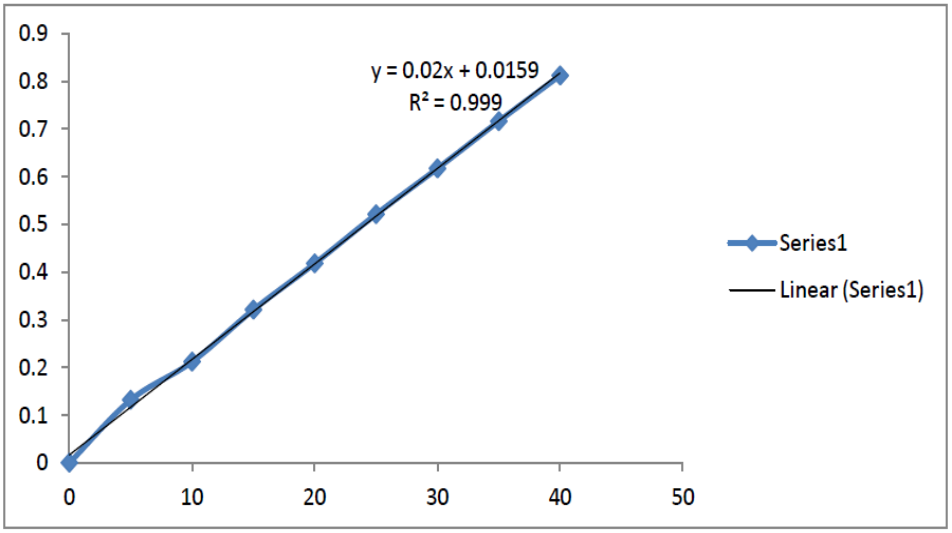

Fig. No. 2: Calibration curve of Terbinafine hydrochloride in ethanol: water (40:60)

Standard curve equation

$y = 0.02x + 0.0159$ $R^2 = 0.999$.

FTIR Studies

FTIR studies were carried out to know the compatibility between drug candidate and excipients. FTIR spectra of the pure drug (Terbinafine hydrochloride) and formulated Proniosomal gel containing Terbinafine hydrochloride shown in the fig no. 6.3 and 6.4 respectively.

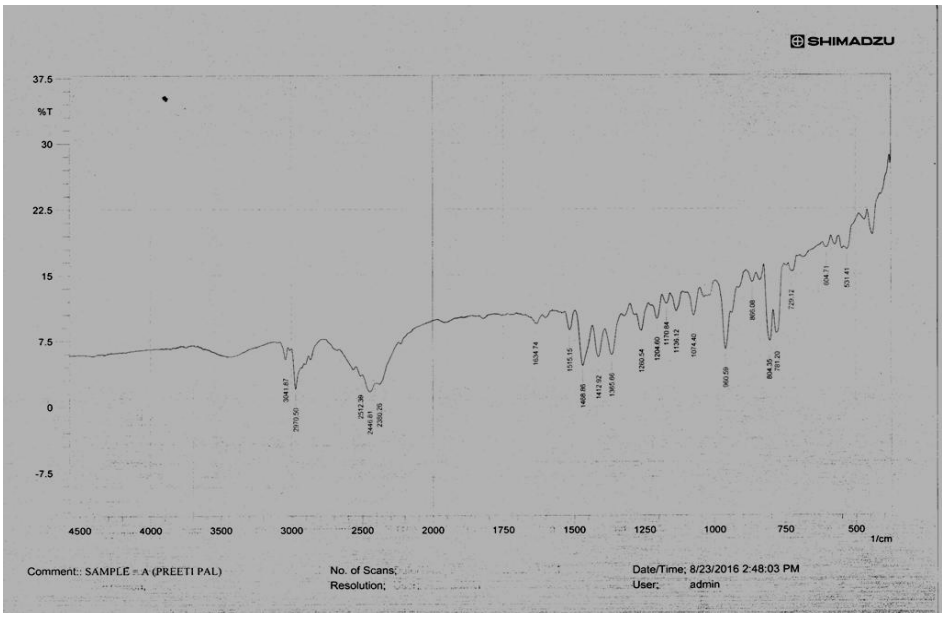

Fig. No. 3: FTIR Spectra of pure Terbinafine hydrochloride.

The FTIR spectrum of pure Terbinafine Hydrochloride exhibits following characteristics peaks at-

531.41 cm^{-1}	Benzene ring (out of plane ring bending)
729.12 cm^{-1}	C-H Bending vibration
804.35 cm^{-1}	C-C Streching vibration
11.36.12 cm^{-1}	C-N Streching vibration
1260.54 cm^{-1}	Methylene twisting and wagging vibration
1412.92 cm^{-1}	C≡C Streching vibration
1468.86 cm^{-1}	Methylene scissoring band (δs CH2)
1634.74 cm^{-1}	C=C Streching vibration
2380.26 cm^{-1}	C-H Streching vibration
2970.50 cm^{-1}	C-H Streching vinbration with methyle group
3041.87 cm^{-1}	= C-H Streching vibration

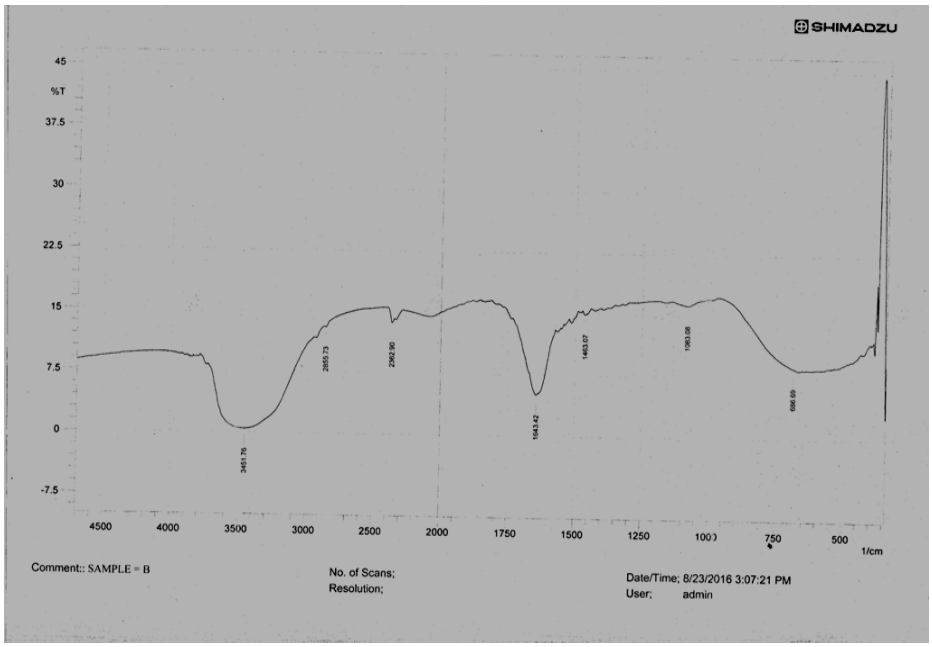

Fig. No. 4: FTIR Spectra of formulated Proniosomal gel

The FTIR spectrum of optimized formulated of Proniosomal gel containing Terbinafine Hydrochloride exhibits following characteristics peaks at-

686.69 cm^{-1}	Out of plane C-H bending vibration
1083.08 cm^{-1}	C-N Stretching vibration
1463.07 cm^{-1}	C≡C Stretching vibration
1643.42 cm^{-1}	C=C Stretching vibration
2362.90 cm^{-1}	C-H Stretching vibration
2855.73 cm^{-1}	C-H Stretching with methyle group

Evaluation of Proniosomal Gel

All prepared batches of proniosomal gel (F1 to F9) were evaluated for the following evaluation parameter to select the optimized formulation as given below.

Physical Evaluation

All the prepared batches (F1to F9) were evaluated visually for colour, consistency, homogeneity and phase separation.

Table No. 6: Physical characterization of the formulated proniosomal gels (F1-F9)

Batches	Colour	Consistency	Homogeneity	Phase separation
F1	Light brown semisolid	Good	homogeneous	None
F2	Yellowish gel	Good	homogeneous	None
F3	Brownish crystalline gel	Fair	homogeneous	None
F4	Yellowish semisolid	Average	homogeneous	None
F5	Yellowish white gel	Excellent	homogeneous	None
F6	Brownish crystalline gel	Good	homogeneous	None
F7	Light yellowish gel	Good	homogeneous	None
F8	Brownish thick gel	Average	homogeneous	None
F9	Offwhite thick gel	Average	homogeneous	None

All the prepared batches of terbinafine hydrochloride proniosomal gel showed optimum physical characters and found to be stable as no phase separation was seen. All batches were homogenous.

Viscosity determination

The viscosity of all the nine formulation ranged from 67402 to 72880 cps. Gel with high viscosity do not easily extrude from the tube whereas, less viscous gels may flow quickly and hence suitable viscosity is required to extrude a gel. The viscosity of F5 (72380 cps) formulation was found to be excellent when compared to other formulation. The results of viscosity of all formulation were shown in table given below.

Table No. 7: Viscosity of all formulated proniosomal gel

Batches	F1	F2	F3	F4	F5	F6	F7	F8	F9
Viscosity (cps)	67327	72035	72745	66854	72380	67521	66402	72880	72364

Percent Entrapment efficiency

The entrapment efficiency of all nine formulations ranged from 65.11 to 90.54%. The percentage entrapment efficiency was maximum when concentration of cholesterol was

250mg and surfactant 1350 mg but on further increasing the concentration of cholesterol and surfactant, the entrapment efficiency of drug decreased. This may be due to the reason cholesterol molecule compete with drug for the space within the Bilayer, remove the drug from the Bilayer and in addition to this disrupt the vesicular membrane structure and mixed micelles formation along with the niosomes vesicles with higher concentration of surfactant and this may lead to lower entrapment efficiency.

Table No. 8: Percent entrapment efficiency of all formulated proniosomal gel

Batc hes	F1	F2	F3	F4	F5	F6	F7	F8	F9
EE (%)	82.14 ±1.10	86.97 ±2.10	88.86 ±1.08	80.07 ±2.30	90.54 ±1.30	70.92 ±1.70	71.60 ±1.50	66.50 ±1.80	65.1 ±0.9

In-Vitro Drug Release Study

Drug diffusion study of all nine formulations was conducted through cellophane membrane. The percentage release of drug from proniosomal gel through a cellophane membrane at various sampling time for 24 hrs was found to be ranging in 66.6% to 95.21% as reported in table no 6.9.

Table No. 9: cumulative % drug release of all formulated proniosomal gels.

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	6.73 ±0.8	8.21 ±1.0	10.52 ±1.0	8.16 ±0.7	10.01 ±1.4	11.34 ±1.5	9.04 ±0.8	8.75 ±0.9	11.18 ±1.9
1	9.60 ±0.8	10.39 ±0.9	14.65 ±0.8	19.57 ±0.7	13.65 ±1.6	15.07 ±0.8	14.62 ±1.3	16.55 ±0.9	17.59 ±1.4
1.5	14.83 ±1.0	19.66 ±1.1	18.41 ±1.4	25.36 ±1.2	18.02 ±1.3	19.97 ±1.4	20.39 ±1.1	22.53 ±0.7	24.62 ±1.4
2	21.04 ±0.9	24.8 ±1.6	24.16 ±1.2	31.86 ±1.0	26.86 ±1.1	25.29 ±1.7	25.79 ±1.1	30.3 ±1.6	33.06 ±1.7
3	25.92 ±1.2	30 ±1.6	30.79 ±1.0	38.04 ±1.4	30.83 ±1.1	31.62 ±2.0	31.67 ±1.5	38.93 ±1.4	44.63 ±1.4
4	32.59 ±1.0	35.85 ±1.1	35.68 ±1.1	46.87 ±1.2	35.73 ±1.6	38.2 ±1.5	37.32 ±1.6	44.5 ±1.4	54.41 ±1.2
5	41.43 ±1.6	43.56 ±1.4	40.89 ±1.3	52.8 ±0.8	43.5 ±0.6	45.04 ±1.5	44.61 ±1.3	50.91 ±1.7	68.28 ±1.5
6	48.49 ±1.4	48.04 ±0.9	44.48 ±1.9	58.39 ±1.2	47.63 ±1.4	55.55 ±2.1	50.81 ±1.6	63.67 ±4.7	73.71 ±2.1

8	55.61 ±0.9	56.91 ±1.6	51.16 ±0.9	65.2 ±0.8	53.74 ±0.9	68.06 ±1.5	56.06 ±1.6	71.85 ±1.3	81.48 ±1.8
10	64.32 ±0.9	67.22 ±1.6	58.88 ±1.3	74.42 ±1.4	62.56 ±2.4	71.37 ±1.8	64.68 ±1.4	81.03 ±1.8	86.65 ±1.5
22	74.27 ±0.9	69.21 ±1.7	67.51 ±1.5	77.73 ±1.2	64.93 ±1.2	85.36 ±1.5	74.68 ±1.6	87.15 ±1.6	92.24 ±1.8
24	77.67 ±1.1	70.62 ±1.5	69.12 ±1.1	79.77 ±1.2	66.6 ±0.9	88.46 ±1.6	84.34 ±1.2	89.71 ±1.3	95.21 ±1.6

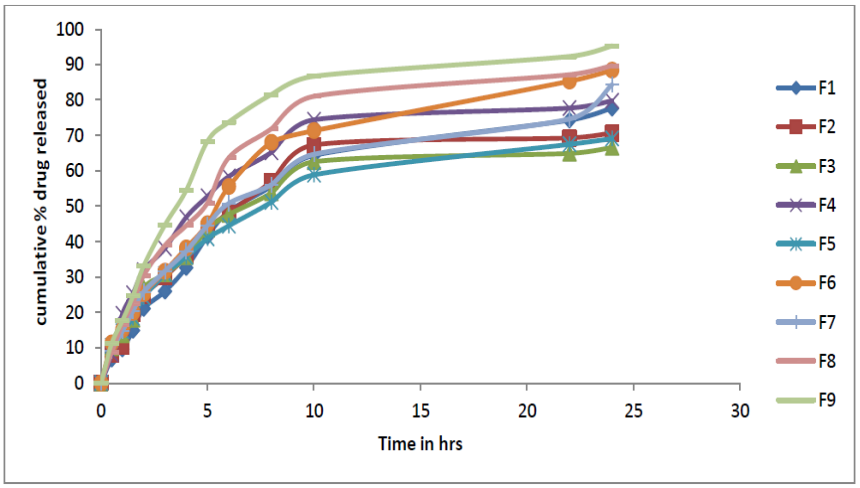

Fig. No. 5: Comparative cumulative % drug release of different formulation of proniosomal gel (F1 to F9)

Vesicles Shape

The result of optical microscopy of optimized proniosomal gel formulation (F5) was showed that the shape of niosomes vesicles derived from Proniosomal gel formulation on hydration with phosphate buffer (ph 7.4) was in spherical as shown in the figure given below.

Fig. No. 6: Optical Microscopy of optimized formulation of proniosomal gel (F5)

Stability studies

The optimized formulation (F5) was found to be stable for period of one month, it can be observed that the gel formulation showed no major alteration in relation to color of formulation, shape of vesicle and entrapment efficiency when it was compared with the freshly prepared proniosomal gel. From the results of stability studies of the optimized formulation (F5) it was concluded that the formulation was stable at refrigerator and room temperature as well.

Table No. 10: Results of stability studies of optimized formulation (F5)

S.No.	Temperature	Initial entrapment efficiency results	After 1 month
1	2º C	90.54	89.56
2	25 ºC	90.54	90.06

DISCUSSION

In this project Tebinafine Hydrochloride was procured as gift sample from Sri Ram Healthcare (P) Ltd, Baddi (Himanchal Pradesh) for the development of antifungal proniosomal gel for topical application.

Firstly Preformulation studies were performed on Terbinafine Hydrochloride and the drug was found to be up to mark.

The analytical method was selected to evaluate the drug concentration in the formulation. For this purpose, the UV method was used to the determination of λ_{max} for drug which was found to be 283 nm and from this a calibration curve was plotted by taking different concentration of solution (5 - 40µg/ml) which can be used as a reference for the in-vitro release studies for Terbinafine hydrochloride by proniosomal gel.

The FTIR studies were performed to know the interaction between different excipients and drug which was used for the formulation of proniosomal gel. FTIR studies result shows that there was no interaction between the drug Terbinafine hydrochloride and excipients used in the formulation of proniosomal gel.

The antifungal proniosomal gel was prepared in nine batches (F1-F9) by using Coacervation phase separation method. These nine batches of proniosomal gel of Terbinafine hydrochloride were prepared by mixing of span 40, cholesterol, soyalecthine as per given

formulation chart.

All batches of prepared proniosomal gel (F1-F9) were evaluated for the following parameters such as physical evaluation, viscosity, entrapment efficiency, in-vitro release, vesicles shape and stability.

All the prepared batches of proniosomal gel (F1-F9) showed optimum physical characters and found to be stable as no phase separation was seen.

The viscosity of all nine batches of proniosomal gel (F1-F9) ranges from 67402 to 72880 cps. The viscosity of F5 formulation (72380 cps) was found to be excellent when compared to other formulation.

The percent entrapment efficiency of all these nine batches of prepared proniosomal gel (F1-F9) was found to be 65.11 to 90.54 with standard deviation between ±0.9 to ±2.3. The percent entrapment efficiency was maximum when used concentration of cholesterol and span 40 was 250 mg and 1350 mg respectively. But on further increasing the concentration of cholesterol and span 40 in the formulation of proniosomal gel, then the entrapment efficiency was decreased. This may be due to the cholesterol molecules compete with drug for the space within Bilayer, remove the drug from Bilayer, and also disruption of the vesicular structure leads to lower entrapment efficiency.

The inverse relationship is existing between the entrapment efficiency and the drug release. Entrapment efficiency is a measure of the vesicle ability to retain the drug; thus, the more the drug is retained in the vesicle, the slower the release profile will be.

The F5 formulation of proniosomal gel was showed the maximum percent entrapment efficiency (90.54±1.3%) and slowest drug release in 24 hrs (66.6±0.9). The slowest drug release indicates the prolonged release pattern of Terbinafine hydrochloride loaded proniosomal gel.

The results of the optical microscopy of F5 formulation proniosomal gel revealed that the niosomes vesicles formed by the hydration of the proniosomal gel were in the spherical shape.

The Terbinafine hydrochloride loaded proniosomal gels were also found to be stable for one

month time period at refrigerator temperature (2ºc - 8ºc) as well as at room temperature (25ºC ± 0.5ºC).

Among all these nine batches of prepared proniosomal gel (F1-F9), F5 formulation was found to be best formulation for further studies due to its prolonged drug release pattern.

CONCLUSION

In this project total nine batches of antifungal proniosomal gel were successfully formulated by co-acervation phase separation method as per formulation table. Terbinafine hydrochloride was used as an antifungal drug to formulate the antifungal proniosomal for topical application. These all nine batches of antifungal proniosomal gel were prepared by mixing the required amount of ingredients (drug, span 40, cholesterol and soya lecithin) as per formulation chart. These all nine batches of prepared proniosomal gel (F1-F9) were evaluated for various parameters such as physical evaluation, viscosity, entrapment efficiency, in-vitro release, vesicles shape and stability.

On the basis of results of evaluation parameters, F5 formulation of proniosomal gel was selected as best formulation out of all nine formulations (F1-F9).

The optimized formulation (F5) was contained Terbinafine hydrochloride (100mg), span 40 (1350mg), soya lecithin (900mg) and cholesterol (250mg). The F5 formulation of proniosomal shows highest % entrapment efficiency (90.54%) and slowest drug release in 24 hrs (66.6%) which indicate the prolonged drug release pattern of formulated proniosomal gel. The F5 formulation of Proniosomal gel was also stable for one month of period and forms spherical shape niosomes vesicles on hydration. Further in-vivo studies can be performed to get the better results of this formulation.

ACKNOWLEDGEMENT

I owe my deep regards to my respected supervisor Dr. Saurabh Mishra (Assistant Professor), Department of Pharmacy, Mahatama Jyotiba Phule Rohilkhand University, Bareilly, for their extraordinary scientific guidance, understanding, and constant encouragement throughout the tenure of my research work.

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