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Formulation and evalution of tolnaftate loaded topical niosomal gel

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Abstract
The aim of the present study was to formulate the topical gel containing tolnaftate niosomes to enhance diffusivity and to facilitate sustained release. The tolnaftate niosomes were prepared by altering the ratios of non ionic surfactants (span60, tween 80, twen60) maintaining cholesterol constant. Thin film hydration technique was employed for this purpose. The prepared niosomes were subjected to entrapment efficiency, size analysis. The obtained niosomes were dispersed into gel which were subjected to *in vitro* diffusion and *exvivo* diffusion studies, microbiological assay. Compared with the marketed available tolnaftate cream the niosomal gel showed higher diffusivity and sustained effect.

Keywords: Tolnaftate, niosomes, *in vitro*, carbopol, exvivo, non ionic surfactants, cholesterol.

1. Introduction
Topical drug delivery system means delivery of API through or in to the skin for direct treatment of cutaneous disorder or the cutaneous manifestation [1]. Conventional preparation like Ointment, gel, cream, emulsion shows problem of drug penetration as well as API deposition. So to overcome this problem there is need to develop novel formulation act as carrier for better penetration through topical formulations [2]. From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, bio recognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology [3].The targeted or site specific delivery of drugs is indeed a very attractive goal because this provides one of the most potential ways to improve the therapeutic index of the drugs [4].
Colloidal drug delivery systems such as liposomes and niosomes have distinct advantages over conventional dosage forms. These systems can act as drug reservoirs and provide controlled release of the active substance. Niosomes are non-ionic surfactant based vesicles that had been developed as alternative controlled drug delivery systems to liposomes in order to overcome the problems associated with sterilization, large-scale production and stability
They are liposome-like vesicles formed from the hydrated mixtures of cholesterol, charge inducing substance, and nonionic surfactants such as mono alkyl or dialkyl polyoxyethylene ether. Basically, these vesicles do not form spontaneously. Thermodynamically stable vesicles form only in the presence of proper mixtures of surfactants and charge inducing agents. The mechanism of vesicle formation upon use of nonionic surfactants is not completely clear. The most common theory is that nonionic surfactants form a closed bilayer in aqueous media based on their amphiphilic nature. Formation of this structure involves some input of energy, for instance by means of physical agitation (e.g. using the hand-shaking method) or heat (e.g. using the heating method) [5-6].
Hydrophilic lipophilic balance & critical packing parameters of non ionic surfactant are important factors during vesicle formulation rather than micelles [7].
Hydrophilic lipophilic balance (HLB) is a good indicator of the vesicle forming ability of any surfactant. If Non-ionic surfactants like polysorbates (tween) are used in formation niosomes have HLB more than 10, thus large concentration of cholesterol requires for stability of niosomes and which in turn results decrease in entrapment efficiency [8].
Gels are transparent to opaque semisolids including high proportion of vehicle to polymer i.e. gelling mediator. Polymer or viscosity modifier adds into suitable vehicle colloidal network was developed. Developed colloidal network restrict flow of fluid by immobilization and trapping of the solvent molecules [9]. Developed niogel preparation having constructive characteristics like greaseless, thixotropic, spreadable, non-staining and it is also compatible

with added ingredients in formulation. present work was done with an objective to formulate Tolnaftate in the form of Niosomal gel, containing emulsion of the drug, with a view to achieve main objective, that is to design a stable topical dosage form of Tolnaftate that will effectively release drug for prolong period time. Tolnaftate is used for patients suffering from athlete's foot, jock itch and ring worm infections. Tolnaftate is also used, along with other antifungals, to treat infections of the nails, scalp, palms, and soles of the feet. It is believed to prevent ergosterol biosynthesis by inhibiting squalene epoxidase. It has also been reported to distort the hyphae and to stunt mycelial growth in susceptible organisms. Tolnaftate is a synthetic thiocarbamate used as an anti-fungal agent. It inhibits the squalene epoxidase [10]. Tolnaftate was found to be only active by topical application and inactive by the oral and intra peritoneal routes of administration [11, 12].

Materials

Tolnaftate was obtained as gift sample from Steamline industries limited, Hyderabad, India. cholesterol, span60, tween 60, tween 80, were purchased from Merck, Mumbai, India. Sodium hydroxide was obtained from SD fine chemicals, Chennai, India.

Methods

Development of Calibration Curve For Tolnaftate: A stock solution of (1mg/ml) of standard drug was prepared, later dilutions were made with ethanol.From this stock solution 10, 20, 30, 40, 50 µg/ml dilutions were prepared using ethanol.

Preparation of Tolnaftate Niosomes

The preparation of niosomes with surfactants, cholesterol and tolnaftate were prepared by dried thin film hydration technique using rotary vaccum evapourator. Accurately weighed drug and other chemicals was dissolved in 10ml of diethyl ether and stirred in mechanical stirrer to form a homogenous mixture. The mixture is dried in rotary evaporator with vaccum of about 25mm Hg at 25^0c.The process was continued until all the diethyl ether gets evaporated and leaves a dried thin film on the surface of the vaccum flask. Then 10ml of PBS pH7.4 was added and hand shaked for half an hour to get a niosomal suspension of multi lamellar vesicles (MLVs).The composition and ratios of surfactant, cholesterol and stabilizers for different types of niosomes were mentioned in the following Table

Table 1: composition of niosomes

S.NO	Formulation	Concentration Of Ingredients	
		Surfactant	Cholesterol
1	$FS60_2$	0.5	1
2	$FS60_3$	1	1
3	$FS60_4$	1.5	1
4	$FS60_5$	2	1
5	$FT80_2$	0.5	1
6	$FT80_3$	1	1
7	$FT80_4$	1.5	1
8	$FT80_5$	2	1
9	$FT60_2$	0.5	1
10	$FT60_3$	1	1
11	$FT60_4$	1.5	1
12	$FT60_5$	2	1

Evaluation of Tolnaftate Niosomes

Morphological characterization

The vesicle formation was confirmed by optical microscopy in 45× resolution. The niosomal suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film of niosomal suspension observed in the formation of vesicles. The microphotography of the niosomes also obtained from the microscope by using a digital camera. The detailed surface characteristic of the selected noisome formulation was observed using a scanning electron microscope.

Preparation of Niosomal Gel

Carbopol 937(2g) was dispersed into purified water under stirring. The pH of carbopol was adjusted to 6.8-7.0 using 2N NaoH.The prepared niosomes were added to carbopol gel under mixing. Finally the pH was adjusted to 6.8-7.0 using 2N NaoH and make up the final volume.

Calibration curve of tolnaftate in UV spectrophotometer

The UV absorbance of tolnaftate standard solutions were in the range of 10-50µg/ml of drug in ethanol showed linearity at 257nm.The linearity was plotted for absorbance(A)against concentration(C)with R^2 value of 0.996 and with the slope equation y =0.018x-0.003.

Characterisation of Niosomes

Percentage of Entrapment Efficiency

Niosomes were centrifuged and the supernatant was diluted with aliquot amount of pH 7.4 buffer and the concentration was determined by UV-Visible spectrophotometer. The amount of drug loaded was determined by the formula:

Entrapment efficiency (%) = $[(C_t - C_f)/C_f]$ 100,

Where, C_t total Drug concentration and C_f free Drug concentration.

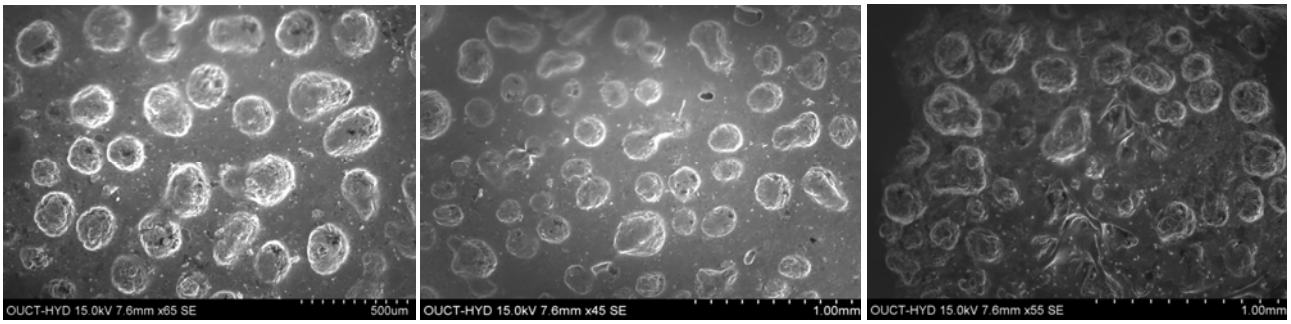
Table 2: Percentage of Entrapment efficiency

S.NO	Formulation	Entrapment Efficiency
1	$FS60_2$	82.99
2	$FS60_3$	83.72
3	$FS60_4$	81.23
4	$FS60_5$	80.07
5	$FT80_2$	77.05
6	$FT80_3$	78.32
7	$FT80_4$	73.28
8	$FT80_5$	69.42
9	$FT60_2$	80.14
10	$FT60_3$	81.76
11	$FT60_4$	79.44
12	$FT60_5$	77.64

Entrapment efficiency of SPAN60 is more compared to Tween 80 and 60. $FS60_3$> $FT60_3$ >$FT80_3$

Determination of particle size

The particle size of the prepared niosomal formulations were determined by using scanning electron microscopy. The size range of prepared nisomal dispersions were noted



SPAN 60 TWEEN 60 TWEEN 80

Fig 1: SEM images of niosomes

Zeta potential

Zeta potential of the dispersion was determined by Malvern zeta meter. Time duration for zeta potential determination was 60 seconds and charge was found out. Zeta potential of formulations are in acceptable range.

Table 3: zeta potential

S. No	Formulation	Zeta Potential
1	$FS60_3$	+13.86
2	$FT80_3$	-12.5
3	$FT60_3$	-13.7

Characterization of Niosomal Gel

Determination of pH

The prepared niosomal gels were measured by a pH meter and the pH of different niosomal preparations was found to be in the range of 6.9 - 7.4

Determination of viscosity

Brookfield DVE viscometer was used for the determination of viscosity of the formulations. About 0.5 g of sample was taken for analysis without dilution the sample by using spindle no. 63 at different rpm at 25±0.5 °C

Table 4: Viscosity of Nisomal gel

S. No	Formulation	Viscosity (Cps)
1	Carbopol gel	63149
2	$FS60_2$	32216
3	$FS60_3$	31260
4	$FS60_4$	31184
5	$FS60_5$	30235
6	$FT80_2$	32418
7	$FT80_3$	31620
8	$FT80_4$	31248
9	$FT80_5$	30360
10	$FT60_2$	31084
11	$FT60_3$	30480
12	$FT60_4$	30220
13	$FT60_5$	29480

Microbiological Assay

Petri dishes containing 20ml medium (Sabouraud dextrose agar) were seeded with 100µl of fungal inoculums (*Aspergillus niger*). The plates were dried at room temperature for 15min.Wells each 2cm in diameter were cut out of the agar.2.2gm of optimized niosomal gel and marketed cream was placed into each well. The fungal plates of *Aspergillus niger* were incubated at 27±0.1 ^{0}c for 2days.The results were recorded by measuring the zone of growth of inhibition surrounding the wells.

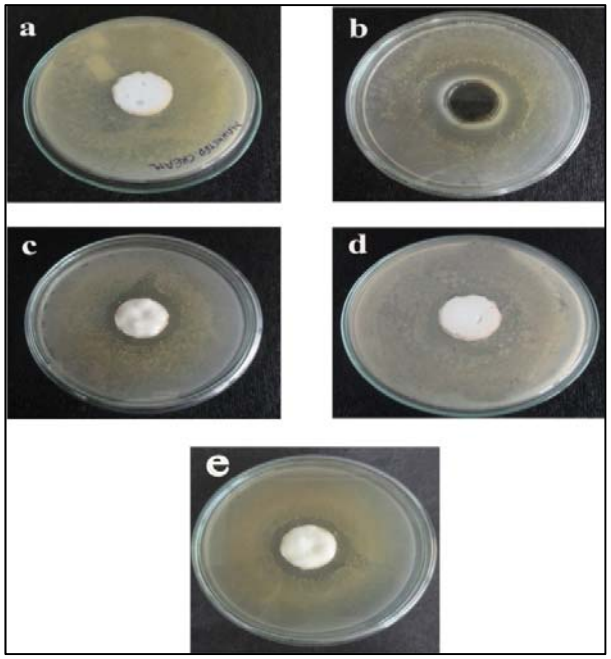


Fig 2: images for zone of inhibition

Table 5: Zone of inhibition

S. No	Formulation	Zone Of Inhibition For 24 Hrs (Cm)	Zone Of Inhibition For 48 Hrs (Cm)
1	$FS60_2$	1.7	3.8
2	$FS60_3$	2.8	5.0
3	$FS60_4$	2.0	3.8
4	$FS60_5$	2.1	4.6
5	$FT80_2$	1.3	2.9
6	$FT80_3$	1.9	3.8
7	$FT80_4$	1.7	3.0
8	$FT80_5$	1.4	3.3
9	$FT60_2$	2.0	3.4
10	$FT60_3$	2.3	4.9
11	$FT60_4$	1.5	3.6
12	$FT60_5$	1.9	4.1

Zone of inhibition of span60 has more than remaining formulation with Tween 80 and Tween 60. Here in these formulation order of Highest zone inhibition is $FS60_3$ > $FT60_3$> $FT80_3$.

***In-vitro* diffusion study**

Diffusion studies were carried out by using franz diffusion cells. Egg membrane was used for this purpose. Membrane

was placed on diffusion cell having 50 ml capacity. Receptor compartment was filled with phosphate buffer solution (pH 7.4) as diffusion medium.
Diffusion assembly was placed on magnetic stirrer and study was carried out at 37 °C ± 2 °C. Test samples i.e. niosomal gel was rest on surface of the membrane. One mL sample was withdrawn at 30 min, 1 hr, 1.5hr, 2 hrs, 3 hrs, 4 hrs, 6 hrs, 8hrs, 10 hrs and 12 hrs time intervals. At each time interval sample withdrawn was replaced with same amount of phosphate buffer solution pH 7.4. Withdrawn samples were analysed for concentration determination using UV spectrophotometry.

Niosomal Gel Formulation Containing Span 60:

Table 6: % drug release profiles of span 60

	Percentage Of Drug Release(N=3)				
TIME	**$FS60_2$**	**$FS60_3$**	**$FS60_4$**	**$FS60_5$**	**Marketed formulation**
0	0	0	0	0	0
0.5	13.27±0.12	10.73±0.09	14.4±0.11	17.23±0.12	7.2±0.04
1	22.03±0.19	16.66±0.11	17.79±0.13	22.31± 0.18	10.4±0.09
1.5	27.1±0.21	20.62±0.16	20.9±0.17	25.7± 0.20	11.4±0.10
2	29.09±0.24	23.72±0.19	25.7±0.21	30.22± 0.25	12.7±0.11
3	32.2±0.29	27.4±0.22	31.92±0.26	34.74± 0.30	14.02±0.10
4	35.31±0.31	36.44±0.31	37±0.31	39.83± 0.32	15.31±0.12
6	37±0.34	40.11±0.34	40.96±0.35	44.63± 0.41	18.42±0.13
8	45.76±0.38	44.06±0.38	44.91±0.39	48.3±0.45	20.18±0.15
10	48.87±0.41	47.17±0.41	50.28±0.41	54.51± 0.48	22.64±0.14
12	53.38±0.45	51.69±0.46	54.8±0.48	61.29± 0.51	24.32±0.17

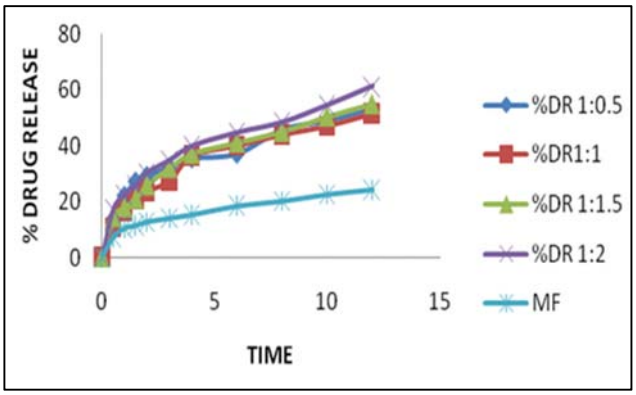


Fig 3: Percentage Drug Release of formulations containing span 60

Based on data $FS60_5$ shows highest percentage drug release. And drug release pattern is Span60>Tween 60>Tween 80.But all the formulations shows better release than marketed Product. $FS60_2$, $FS60_3$, $FS60_4$, $FS60_5$ invitro drug release for 12 hrs is 53.38%, 51.69%, 54.8% and 61.29%. $FT80_2$, $FT80_3$, $FT80_4$, $FT80_5$ drug release for 12 hrs is 43.5%, 42.09%, 44.35% and 46.01% respectively. $FT60_2$, $FT60_3$, $FT60_4$, $FT60_5$ drug release for 12 hrs is 44.35%, 42.09%, 45.48% and 48.3%. Marketed product drug release for 12 hrs is 24.32%

***Ex- vivo* diffusion study**

Franz diffusion cell was used to carry out *Ex-vivo* skin permeation studies. For that rat skin was used as model membrane. Initailly rat skin was shaved to remove the fat carefully and washed with the buffer. Shaved skin was placed on diffusion cell having capacity 50 mL. Receptor compartment is filled with phosphate buffer solution (pH 7.4) as diffusion medium. Dermal side of the skin was kept facing to receptor side and epidermal surface towards donor compartment.
Diffusion assembly was placed on magnetic stirrer and study was carried out at 37 °C ± 2 °C. Test samples i.e. niosomal gel was rest on dermal side separately. One mL sample was withdrawn at 30 min, 1 hr, 1. 5hr, 2 hrs, 3 hrs, 4 hrs, 6 hrs, 8hrs, 10 hrs and 12 hrs time intervals. At each time interval withdrawn sample was replaced with same amount of phosphate buffer solution pH 7. 4. Withdrawn samples were analysed for concentration determination using UV spectro photometry.

Niosomal Gel Formulation Containing Span 60

Table 7: % drug release profiles of span 60

Percentage Drug Release					
Time	**$FS60_2$**	**$FS60_3$**	**$FS60_4$**	**$FS60_5$**	**Marketed formulation**
0	0	0	0	0	0
0.5	12.14±0.07	9.8±0.04	12.71±0.05	15.25±0.07	6.4±0.02
1	17.23±0.09	14.6±0.08	16.37±0.10	20.62±0.12	9.3±0.05
1.5	19.20±0.11	18.9±0.12	19.20±0.11	24.01±0.17	10.6±0.08
2	23.16±0.14	20.05±0.15	25.14±0.14	26.27±0.21	11.3±0.08
3	28.81±0.18	26.83±0.20	30.22±0.13	29.94±0.25	13.5±0.07
4	33.05±0.22	30.52±0.26	36.15±0.24	37.57±0.29	15.6±0.11
6	36.44±0.26	38.9±0.31	38.70±0.27	40.96±0.31	17.4±0.10
8	43.22±0.31	41.52±0.34	42.93±0.26	47.74±0.30	19.78±0.13
10	46.61±0.34	43.22±0.38	48.30±0.32	52.26±0.35	21.22±0.14
12	51.13±0.39	50.56±0.36	52.82±0.42	59.60±0.41	23.18±0.17

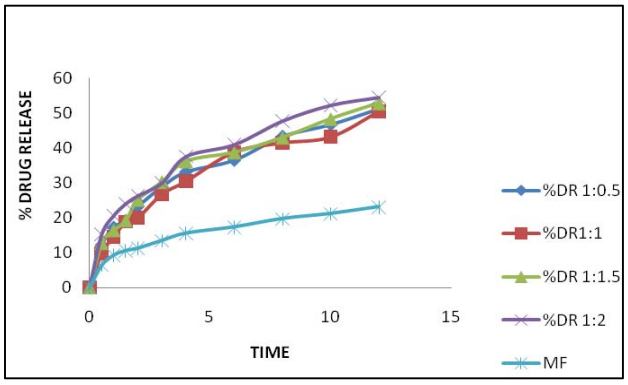


Fig 4: percentage Drug release of formulations containing span 60

Based on data $FS60_5$ shows highest percentage drug release. And drug release pattern is Span60>Tween 60>Tween 80.But all the formulations shows better release than marketed Product. $FS60_2$, $FS60_3$, $FS60_4$, $FS60_5$ drug release for 12 hrs is 51.13%, 50.56%, 52.82% and 59.60%. $FT80_2$, $FT80_3$, $FT80_4$, $FT80_5$ drug release for 12 hrs is 42.65%, 40.96%, 43.22% and 45.48% respectively. $FT60_2$, $FT60_3$, $FT60_4$, $FT60_5$ drug release for 12 hrs is 43.78%, 35.02%, 44.06% and 46.04%. Marketed product drug release for 12 hrs is 24.32%.

Compatibility studies

The compatability between the drug and selected surfactants and other chemicals were evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-surfactant mixture, which confirmed the absence of any chemical interaction between the drug, surfactant and other excepients.

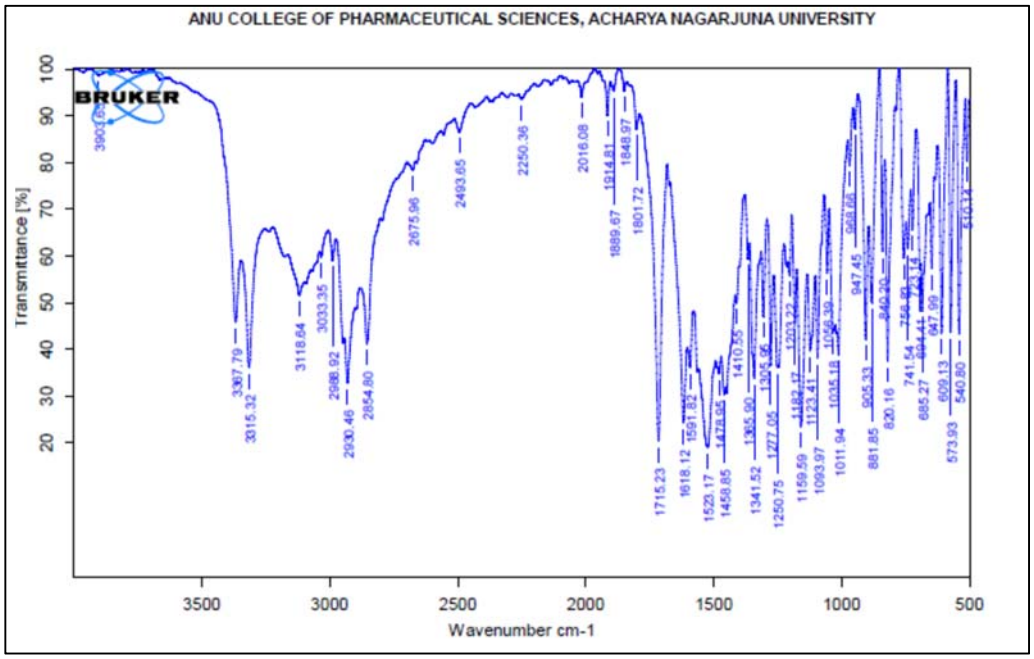


Fig 4: FTIR of Tolnaftate

Conclusion

Niosomes are recognized as novel drug delivery systems. Niosomes can accomo date drug molecule with a wide range of solubilities because of the presence of hydrophilic and hydrophobic moieties together in their structure.

Niosome carrier released the active pharmaceutical ingredient in sustainable manner and develops niosomal delivery system for fungal infection. Afterwards niogel was developed for improvement of topical applicability of niosomal dispersion to localize API at target site of action.

These studies demonstrated that the low soluble Tolnaftate drug could be entrapped between the niosomal structure having both hydrophilic and hydrophobic moieties. Changes in the micromolar ratios of nonionic surfactant with a constant ratio of cholesterol are associated with the changes in the entrapment and release rates of Tolnaftate from SPAN 60, TWEEN 80 and TWEEN 60 niosomes.

Based on the results obtained from the evaluation parameters niosomes formulated with 1:1 molar ratios of surfactants and cholesterol showed highest entrapment efficiency and good sustained effect. The formulations $FS60_3$, $FT80_3$, $FT60_3$ showed entrapment efficiency of about 83.72%, 78.32%, 81.76%.

The prepared formulations showed high release rates than that of marketed formulation (24.32%). Hence it can be concluded that a suitable topical applicable niosomal gel with better results was developed for treatment of fungal disease.

All the formulations though they possesed good release patterns, formulations $FS60_3$, $FT80_3$, $FT60_3$ showed sustained effect compared to other formulations. In-vitro release from egg membrane showed 51.69%, 42.09%, 43% respectively for 12 hrs while ex- vivo diffusion from rat skin membrane showed 50.56%, 40.96%, 35.02% release respectively for 12 hrs compared with that of marketed cream which is 24.32% release for 12 hrs.

That is 1:1 molar ratio of surfactant and cholesterol showed not only good release profile but also good sustained effect.

The various preformulation studies like solubility; melting point of the drug has been performed in order to know the purity of the drug. The results of all the performed formulation studies like pH, viscosity, SEM, zeta potential, microbiological assay, in-vitro and ex-vivo diffusion etc were

shown good results. $FS60_3$ Formulation has good entrapment efficiency, zeta potential, Zone of inhibition and better sustainability.

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