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DEVELOPMENT AND CHARACTERIZATION OF TOPICAL LIPOSOMAL GEL BEARING FLUCONAZOLE

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ABSTRACT

The objective of this study was to develop aFluconazole liposomal gel intended for anti-fungal activity compared to a Normal gel. The liposomes constituting of soya lecithin, cholesterol and FLZ were prepared by thin film hydration technique. The prepared liposomes were characterized for vesicle size, shape, morphology, zeta potential, encapsulation efficiency and in-vitro drug release. The percentage of FLZ entrapped in all liposome formulation varied between 60.8 to 78.12%. Gel containing liposomal dispersion was prepared in Carbopol 934 and characterized for gel strength, viscosity, pH and content uniformity. Stability studies were performed for both liposomal dispersion and gel formulation for 3 months. Amongst different storage conditions, the liposomes stored at 2 to 8°C were found to be most stable, with only 5% drug loss over the storage period of 5 weeks. Significantly higher drug permeation of liposomal formulation has been achieved as compared to plain drug solution and carbopol containing FLZ.

INTRODUCTION

Liposomes are microscopic lamellar structure formed on the admixture of Soya lecithin, Cholesterol, Tocopherol acetate with subsequent hydration in aqueous media. Liposomes have been widely evaluated for Controlled and targeted drug delivery for treatment of cancer, viral infections and other microbial diseases. Liposomes are found to be suitable for localization of topically applied drugs at or near the site of application, due to fact they may act as slow releasing vehicles. [1] Fluconazole is a broad spectrum anti fungal agent active against a wide variety of fungi and yeast. It is rapidly but incompletely absorbed after oral dosing and is highly variable. Topically it is used in the treatment of Candida or tinea infections of skin. Encapsulation of FLZ in liposomes may increase the half life providing prolonged drug delivery and minimize the commonly occurring side effects. [2, 3, 4, 5] The objective of present work is to prepare FLZ liposomal gel and study the in-vitro release and stability studies of prepared liposomes.

MATERIALS AND METHODS

Fluconazole was a generous gift from Fourrts India Private Limited (Chennai, India). Lecithin were purchased from Hi Media (Mumbai, India), Cholesterol were purchased from Central Drug House (Mumbai, India). Carbopol 934 was kindly donated from Lubrizol, Cleveland and Dialysis membrane

were purchased from Hi Media (Mumbai, India).Potassium dihydrogen orthophosphate and Sodium hydroxide were purchased from Central Drug House (Mumbai, India).Methanol was purchased from Merck Pvt. Ltd. (Mumbai, India). All other chemicals were of analytical grade.

Liposomes preparations

Liposomes were prepared by thin film hydration technique [6, 7, 8] using rotary flash evaporator. FLZ; SPC (Soya Phosphotidyl Choline): CHOL ratios were altered and drug entrapment efficiency was studied. Briefly a chloroform: methanol (2:1) mixture of FLZ: SPC: CHOL (10: 100: 05 W/W/W) was first dried completely in rotary evaporator under vacuum at 40^0 C to form a lipid film was then hydrated with distilled water for 2 hrs at 37^0 C. the preparation was sonicated at 4°C in three cycles of the 5 min and rest of the 5 min between each cycle by using probe sonicator. The formation was homogenized at 15,000 psi pressure in three cycles using high pressure homogenizer to get liposomes.

FLZ vesicle cauterization

Visualization by transmission electron microscopy (TEM)

TEM analysis was used to examine the ultra structure of liposomes. To prepare samples, copper grids were coated with a solution of collodion and then a drop of liposomal dispersion was applied and left in contact for 15min. finally, grids were picked up, blotted with filter paper, left to dry for 3min and then analyzed with TEM[9, 10, 11].

Vesicle size and zeta potential

The average diameter of the vesicle and their zeta potential where determined by using a zeta master apparatus (Malvern Instruments, Malvern, UK) at a temperature of 25o ± 0.1°C.[12, 13, 14]

Drug entrapment efficiency

The liposome suspension was ultra centrifuged at 5000 rpm for 15 minutes at 4°C temperature by using remi cooling centrifuge to separate the free drug. A supernant containing liposomes in suspended stage and free drug at the wall of centrifugation tube. The supernatant was collected and again centrifuged at 15000 rpm at 4°C temperature for 30 minutes. A clear solution of supernant and pellets of liposomes were obtained. The pellet containing only liposomes was resuspended in distilled water until further processing. The liposomes free from unentrapped drug were soaked in 10 ml of methanol and then sonicated for 10 min. The vesicles were broken to release the drug, which was then estimated for the drug content. The absorbance of the drug was noted at 261.4 nm. The entrapment efficiency was then calculated using following equation[15].

% Entrapment efficiency= Entrapped drug /Total drug added ×100

Preparation of Carbopol gels

As a vehicle for incorporation of liposomes for topical delivery, a carbopol gel was made. Carbopol 934 (1 g) was dispersed in distilled water (88 g) by stirring at 800 rpm for 60 minutes. Then, propylene glycol (10 g) was added and the mixture was neutralized by drop wise addition of tri methanol amine. Mixing was continued until a transparent gel appeared[16, 17, 18].

Incorporation of liposomes of optimized batch into carbopol gel

Liposomes containing FLZ (separated from the unentrapped drug) were mixed into the 1% (w/w) Carbopol gel with an electrical mixer (25 rpm, 2 min), the amount of liposomes of optimized batch (F7) added into the gel, such that the prepared gel have 1% w/w FLZ concentration 10 mg drug per 1gm of gel[19].

EVALUATION OF LIPOSOMAL TOPICALGEL

Physical examination

The prepared gel formulations were inspected visually for their color, homogeneity, consistency, and spread ability[20].

P^H [21]: The pH values of 1% aqueous solutions of the prepared gels were measured by a pH meter

Viscosity: Viscosity of prepared gels were measured by Brookfield-DV-II+Pro Viscometer. Apparent viscosity measured at 25°C and rotating the spindle at 1.5 rpm.

Content uniformity: Gel formulations (100 mg) was dissolved in methanol and filtered and the volume was made to 100 ml with methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 261.4 nm using UV Visible spectrophotometer.

***In vitro* Drug Diffusion Study of liposomal gel:** Skin permeation studies with Fluconazole containing liposomal formulations (liposomes

incorporated in Carbopol gel), were carried out using dialysis membrane employing in open ended cylinder[22]. Liposomal or non-liposomal Fluconazole formulation amount equivalent to 10 mg of drug) was applied uniformly on the dialysis membrane. Aliquots of 2 ml were withdrawn periodically and replaced with same amount of phosphate buffer pH 7.4 to maintain the receptor phase volume at a constant level. The samples were quantified spectrophotometrically at a λ max of 261.4 nm.

STABILITY OF LIPOSOMES: The stability of a pharmaceutical delivery system may be defined as the capability of a particular formulation, in a specific container to remain within its Physical, chemical, microbiological, therapeutic and toxicological specification. Based on entrapment efficiency, the optimized formulation F7 was selected for stability studies. The selected formulation was stored in light protected glass bottle at 4 ℃, 37° C and 45℃ over a period of 60 days. After 30 days and 60days time interval the formulations were viewed under microscope, the aggregation was noted.

RESULTS AND DISCUSSIONS

FLZ loaded liposomes were prepared by thin film hydration technique by using rotary flash evaporator. The liposomes were prepared by using different concentration of FLZ, SPC and CHOL, Methanol and water. The composition of different liposomal formulation prepared in this investigation is recorded.

Characterization of liposomes:

The obtained liposomes formulations were characterized for particle vesicles size, zeta potential and entrapment efficiency. The particle vesicles sizes of all formulations were found to be the range between 90.96 nm to 303.1 nm. The percentage entrapment efficiency of all liposomal batches were found to be 61.87% to 78.1% the maximum entrapment was observed in batch F7 and the particle size of F7 batch was found to be 136.6 nm. Based on the above findings F7 formulations were selected for further studies. The particle size range of F7 formulation was found to be 136.6 nm.

The liposomes were found to be spherical vesicles when examined by TEM after negative staining with phosphotungstic acid. Liposomes are multilamellar vesicles with mean size of 50 to 200 nm. (Fig: 2)

EVALUATION OF LIPOSOMAL TOPICALGEL

i) Physical examination: The prepared gel formulations were inspected visually for their color, homogeneity, consistency, and spread ability.

ii) P^H: The pH values of prepared liposomal gel were 7.2

iii) Viscosity: The measured viscosity of prepared liposomal gel were 977.5cps

IV) Content uniformity: Drug content of prepared liposomal gel were 87.5%

V) *In-vitro* drug release:

The liposomal gel formulation followed zero-order, first-order, matrix, Higuchi and Korsmeyer-Peppas models, though the latter was the best fit model. From the calculated values of coefficients of determination, it can be concluded that drug release from this formulation mostly following the matrix kinetics. This formulation is showing good correlation with Korsmeyer-Peppas release model. Therefore, drug release mechanism can be predicted from the calculated values of release rate exponent (n). The value of 'n' is 0.5356. Therefore, it can be concluded that according to Korsmeyer-Peppas model drug release is mainly following Anomalous Transport which corresponds to diffusion. The relative complexity of these formulations indicated that the drug release was controlled by more than one process. The amount of Fluconazole permeated in 8 hrs was found to be 75.02% and 72.33% from liposomal suspension and liposomal gel, respectively whereas only 35.82% and 28.45% of the drug permeated in case of aqueous solution and carbopol gel, respectively. Clearly vouch for the permeation enhancing effect of vesiculation on the drug.

STABILITY OF LIPOSOMES:

Effect of storage on aggregations:

The liposomal final formulation was stored in light protected glass bottle at 4℃, 37° C and 45℃ over a period of 60 days. After 30 days and 60days time interval the formulations were viewed under microscope, the aggregation data was obtained and presented in table.

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (r.nm): 136.6	Peak 1:	97.72	57.9	51.31
PdI: 0.487	Peak 2:	553.8	31.0	293.8
Intercept: 0.891	Peak 3:	2104	11.2	491.1
Result quality : Good				

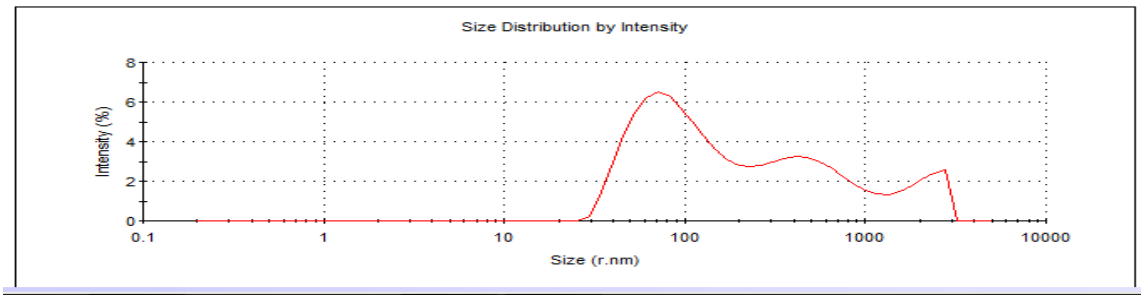

Fig 1. Particle size of liposomal F7 formulation

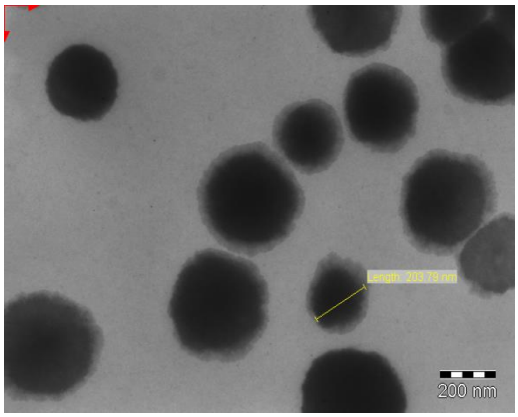

Fig 2. TEM photographs of Liposomal Formulation

Table 1. Formulation of Fluconazole liposomes by thin film hydration technique

Formulation	Fluconazole (mg)	Lecithin(mg)	Cholesterol(mg)	Chloroform (ml)	Methanol(ml)	Water (ml)
F1	10	100	05	2	1	5
F2	10	100	10	2	1	5
F3	10	100	15	2	1	5
F4	10	100	20	2	1	5
F5	10	100	30	2	1	5
F6	10	100	40	2	1	5
F7	10	100	50	2	1	5
F8	10	100	60	2	1	5
F9	10	100	70	2	1	5

Table 2. Particle size, Zeta potential, Entrapment efficiency of liposomes

Formulation	Particle vesicles size(nm)	Zeta potential	Entrapment efficiency
F1	90.96	-66.2	70.12 ± 0.61
F2	109.6	-64.7	65.12 ± 1.10
F3	110.9	-67.0	61.87 ± 0.63
F4	115.4	-69.9	68.25 ± 0.65
F5	122.3	-79.1	71.37 ± 0.86
F6	135.1	-70.3	64.25 ± 0.92
F7	136.6	-69.6	78.12 ± 0.40
F8	297.5	-66.2	62.37 ± 0.51
F9	303.1	-66.3	60.87 ± 1.02

Table 3. Preparation of carbopol gel

S. No	Ingredients	Quantity (gm)
1	Carbopol	1
2	Propylene glycol	10
3	Water	88
4	Tri ethanol amine	Q.S

Table 4. Diffusion profile of liposomal gel

Time (hr)	% Cumulative drug release (Mean± S.D)
0	0.00
1	30.33±0.42
2	38.15±0.16
3	43.52±0.26
4	48.88±0.38
5	54.57±0.76
6	60.60±0.30
7	66.60±0.52
8	72.33±0.48

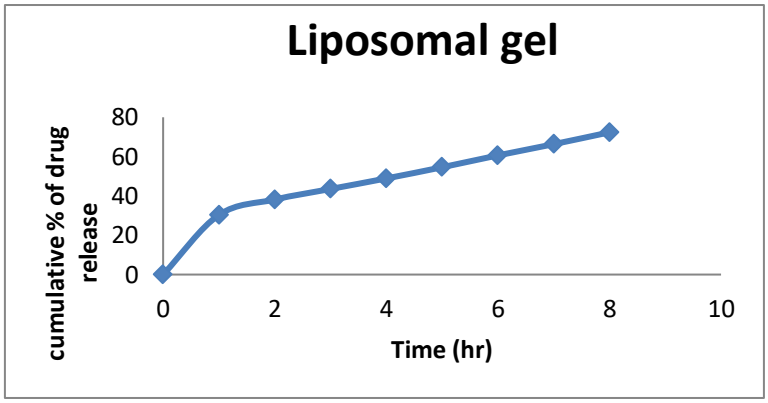

Fig 3.Cumulative % of drug release of liposomal gel formulation

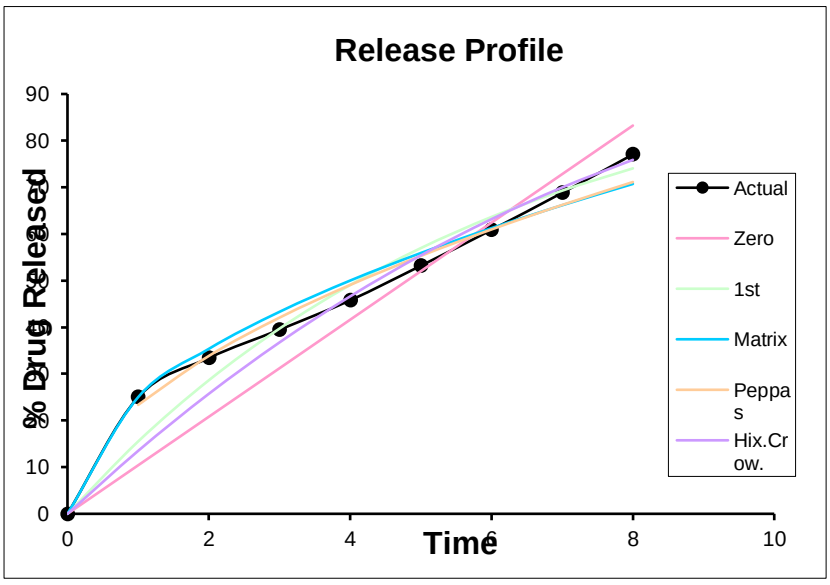

Fig 4. Drug Release Kinetics of liposomal gel formulation

Table 5. Kinetic models of liposomal gel

Kinetic models	R	K	N
Zero order	0.9410	10.4039	-
1st order	0.9847	-0.1687	-
Matrix	0.9898	0.9847	-
Peppas	0.9886	23.3502	0.5356
Hixson Crowell	0.9836	-0.0472	-

Table 6. Comparative drug releases of different formulations

Time(hr)	% Cumulative drug release Mean± S.D			
	Aqueous solution	Carbopol gel	Liposomal suspension	Liposomal gel
0	0.00	0.00	0.00	0.00
1	9.66±0.52	6.66±0.23	33.66±0.76	30.33±0.42
2	12.71±1.04	9.7±0.53	41.83±0.52	38.15±0.16
3	15.73±0.86	13.71±0.28	49.20±0.46	43.52±0.26
4	20.07±0.93	17.73±0.74	55.57±0.83	48.88±0.38
5	26.10±0.25	20.42±0.82	62.94±0.32	54.57±0.76
6	28.46±0.64	22.43±0.65	66.98±0.20	60.60±0.30
7	32.47±0.46	24.44±0.34	71.00±0.58	66.60±0.52
8	35.82±0.38	28.45±0.72	75.02±0.84	72.33±0.48

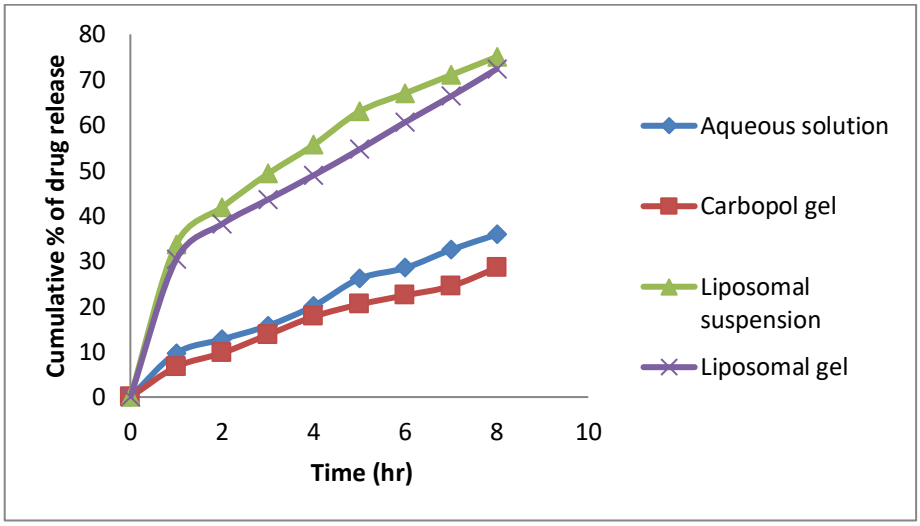

Fig 5. Comparative drug releases of different formulations

Table 7. Effect of storage on vesicle aggregation

After 30 days			After 60days		
4° C	37°C	45°C	4°C	37°C	45°C
-	-	+	-	+	++

Note: + - aggregates of below 5 vesicles; ++ - aggregates of 5-10 vesicles

CONCLUSION

Liposomal formulation of Fluconazole gel was found to have reasonable drug loading, controlled release rate, particle size and stability and phase transition behavior. The formulated FLZ liposomes gel have shown an appreciably enhanced retention of drug molecule in the skin. Thus the liposomal gel formulation, with desired characteristics for administration, could be successfully prepared.

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