Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2015, 6(5):61-66

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Formulation, characterization and evaluation of fluconazole liposomes

Devi R.[1], Prasad C. M.[2], Renganathan A.[2], Kasthuri S.[1], Sundhararajan R.[1] and Deepa N.[1]

[1]Mohamed Sathak A. J College of Pharmacy, Sholinganallur, Chennai
[2]Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi

---

ABSTRACT

The aim of this work was to prepare and evaluate the topical liposomes of Fluconazole. Fluconazole loaded liposomes were prepared by thin film hydration technique. The prepared liposomes were evaluated for in-vitro drug release. The in-vitro diffusion study was carried out by dialysis membrane using both open ended tube. The study was carried out in 40 ml of phosphate buffer solution pH 7.4. The percentage cumulative release from the optimized batch i.e. F7 with drug: lecithin: cholesterol ratio 1: 10: 5, found to be 75.02% release in 8 hours. The magnitude of drug retention within the vesicles on storage under defined conditions ultimately governs shelf life of the developed formulations. Liposomes showed an increasing vesicle size in accelerated temperature but no significance changes at 4 ±2^{0}C has observed in storage studies for two months

Key words: Antifungal, Liposomes, Fluconazole, Soya lecithin, Cholesterol.

INTRODUCTION

Fluconazole (FLZ) is a broad- spectrum antifungal agent used for dermal infection caused by various species of pathogenic dermatophytes[1, 2].It is used for the treatment of Candidiasis. Candidiasis is an infection caused by Candida, yeast like fungus. Candidiasis usually affects the skin and mucous membranes (soft, moist areas around body openings like mouth and anus). Children's of any age develop Candida paronychia an infection of the skin around the nails. Older girls and woman may develop Candida vulvavaginitis an infection of the vagina and the area around the vaginal opening. A number of antifungal creams are now in use and employed for a variety of dermatological and other mycotic infections. However many types of such fungal z1infections have proven to be persistent and defeat any attempts to control or cure them. In addition, local reaction including irritation and burning sensation may occur in patients treated topically.

In dermal and transdermal delivery the skin is used as a portal of entry for drugs for localized and systemic treatment[3, 4].Because of the barrier property of the outer layer of the skin in many cases, permeation enhancing agents are needed to achieve therapeutic level of drug.[5] Classic liposomal systems were found to be effective at forming drug reservoir in the upper layer of the skin, for local skin therapy [6].

Liposomes are microscopic lamellar structure formed on the admixture of soya-lecithin cholesterol. The liposomal encapsulation of FLZ is increases skin resistance time leading to faster healing to external lesions and to a reduction of side effects and duration of therapy.

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, Clevelandand 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.

Preparation of MultilamellarVesicles[7, 8]
The liposomes used in this study were prepared by simplest and most widely used method of mechanical dispersion commonly known as thin film hydration method. In order to prepare better engineered liposome, it was advised to start with a round bottom glass vessel of large volume, so that large surface area was produced to form a very thin film. Drying down temperature was regulated just above the phase transition temperature of the lipids used. In addition to this, on using glass beads (0.5-3.3mm in diameter) offered very effective aid for dispersion of the lipid. Lipid mixture containing PhosphotidylCholine, cholesterol in different molar ratios was dissolved in minimum amount of chloroform (2:1 ratio) in a round bottom flask. To the above solution, 1ml methanolic solution of Fluconazole was added. The organic solvent was removed using rotary flask evaporator at 40°C under vacuum 250 mmHg. The organic solvent was slowly removed by this process such that a very thin film of dry lipids was formed on the inner surface of the flask. The dry lipid film was slowly hydrated with 5ml of distilled water. The flask was once again rotated at the same speed as before and at room temperature for 1 hr. The liposomal suspension was left to mature overnight at 4ºC, to ensure full lipid hydration.

Liposomes preparations
Liposomes were prepared by tin film hydration technique[9, 10,11] 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 vaccum 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^oC 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)[12, 13, 14]
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.

Vesicle size and zeta potential[15, 16, 17]
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 $25^o \pm 0.1^o$C.

Drug entrapment efficiency[15]
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 supernant 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.

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

In -vitro **drug diffusion studies**[18, 19,20]

The release of drug was determined by using the dialysis membrane mounted on the one end of open tube, containing 5ml of liposomal suspension (containing 10 mg of FLZ). The dialysis tube was suspended in 100ml beaker containing 40 ml of PBS (pH 7.4).The solution was stirred at 200rpm with the help of magnetic stirrer at room temperature .perfect sink conditions were maintained during the drug release testing. The sample were withdrawn at suitable time interval at (1,2,3,4,5,6,7,8 h).The dissolution medium was replaced with same amount of fresh PBS(pH 7.4) solution to maintain the volume 40ml throughout the experiment. The drug content in the withdrawn samples(2ml)were estimated at 261.1nm after making the volume up to 10 ml PBS (pH 7.4)and cumulative % of drug released was calculated and plotted against time (t).

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 °C, 37° C and 45°C 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 DISCUSSION

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.

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

Characterization of liposomes:

The obtained liposomes formulations were characterized for particle vesicles size, zeta potential and entrapment efficiency.

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

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.

		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				

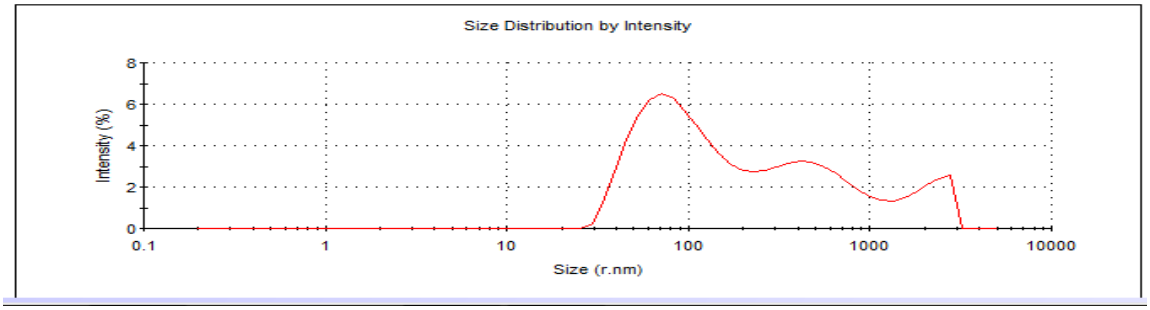

Fig: 1. Particle size of liposomal F7 formulation

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)

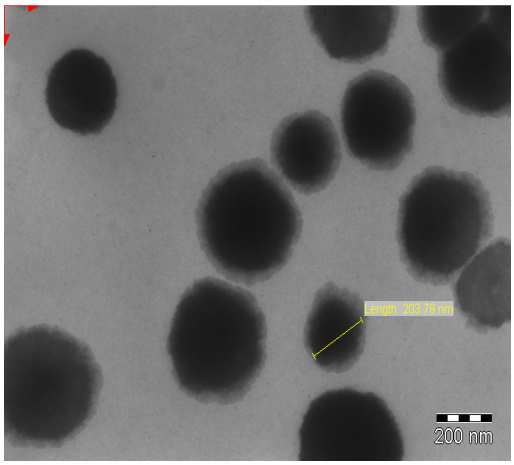

Fig: 2 TEM photographs of Liposomal Formulation

In-vitro drug diffusion studies

The cumulative amount of drug permeated across dialysis membrane was plotted as a function of time and % drug release was calculated from the slope of linear portion. The release profile was shown in the figure 3.In all the formulations, formulation F7 with drug: lecithin: cholesterol ratio1:10:5 was foundto give a better result i.e.; drug 75.02% release in 8 hours.

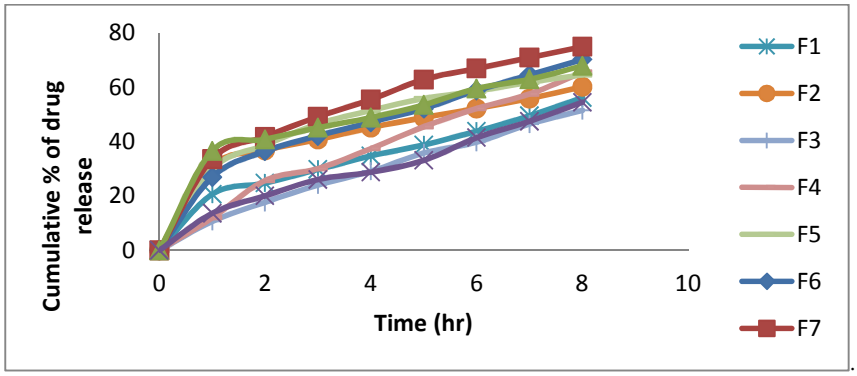

Fig: 3 Comparative diffusion studies of all batches

The amount of FLZ permeated in 8 hrs was found to be 75.02% from liposomes, 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 the drug

Table: 3.Comparative drug release of different formulations

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

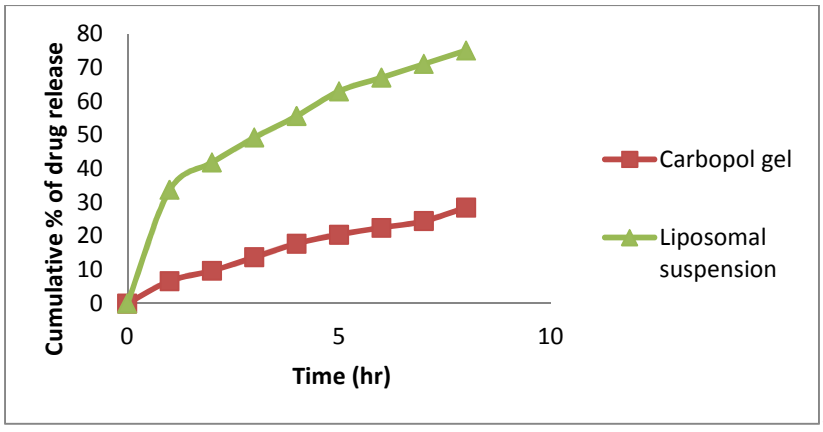

Fig: 4 Comparative drug releases of different formulations

Stability of liposomes

The stability of vesicles to retain the drug was assessed by keeping the liposomal suspension at four different temperature conditions i.e., 4 -8^{o}C(Refrigerator), 25±2 ^{o}C (Room temperature), 37±2 ^{o}C and 45±2 ^{o}C over a period of 60 days. After 30 days and 60 days time interval the formulations were viewed under microscope, the aggregation data was tabulated.

Table: 4 Stability studies of Liposomes at different temperature.

After 30 days				After 60days			
4-8°C	25±2°C	37±2°C	45±2°C	4-8°C	25±2°C	37±2°C	45±2°C
-	-	-	+	-	-	+	++

\+ *- aggregates of below 5 vesicles*
++ *- aggregates of 5-10 vesicles*

The magnitude of drug retention within the vesicles, on storage under defined conditions, ultimately governs shelf life of the developed formulations. Liposome showed an increasing vesicle size in accelerated temperature but no significance changes at 4±1°C has observed in storage studies for two months.

CONCLUSION

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

Acknowledgement
We are thankful to Fourts India Pvt Ltd, Chennai for providing gift sample of Fluconazole, the author would like thank to dean and management of BIT, Mesra, Ranchi for providing necessary facilities useful in conduction of this work.

REFERENCES

[1] Naik A,kalia Y N, Guy R H,*PSTT*.**2000**,3, 318.
[2] Schaefer H & Redelmeier T E, *Skin barrier, principles of percutaneous absorption* (karger, Basel) **1996**, pp 235.
[3] Bouwstra J.A. Honey well- Nguyen P L. Gooris G. S &Ponee M,*Prog lipid Res*.**2003**, 42,1.
[4] Geve G,*Cri Rev Therapeutic Drug Carrier Sys*.**1996**, *13*, pp 257.
[5] Ceve G&Blume G, *Biochim Biophys Acta*.**1992**, 226,*1104*.
[6] Ceve G &Blume G,*Biochim Biophys Acta*.**2001**, 191.1514.
[7] Avinashkumar Seth. Mathematical modelling of preparation of acyclovir liposomes,reverse phase evaporation method. *J .Pharm. Sci* **2002**,285-291.
[8] Wen, A.H, Choi1, M.K, & Kim, D.D,*Arch. Pharm. Res*.**2006**, 29(12), 1187-1192.
[9] Amit Bhatia, Rajiv kumar,*J .Pharm. Sci*.**2005**,7,(2),252-259.
[10] Rakesh PatelP, HartickPatel, *Int. of drug deliv .tech*.**2009**, 16-23.
[11] Jaafari M.R, Malaekeh B, *IJBMS*.**2005**, 8(2), 195-201.
[12] Bhalaria M.K, Sachin N,Misra A.N,*Indian J. of Exp.Biology*.**2009**, 47,368-375.
[13] Lopez-pinto J.M, *Int. J. of phar.sci*.**2005**, 298,1-12.
[14] Ning M, Guo Y, Pan H, Chen X.&Gu Z,*Drug development and industrial pharmacy*.**2005**, 31, pp 375-383.
[15] Rakesh P, Hartick P,*Int.J of drug deliv .tech*.**2009**, 16-23.
[16] Paola M, Francesca M, *Eur.J.of pharmaceutics and Bio pharmaceutics*.**2007**, 86-95.
[17] AgarwalR, Katare O.P,*Pharmaceutical Technology*.**2002**, pp 48-60.
[18] Vandana Gupta, Ashok B,Suman R, Ind .*J.of Pharm sci*,**2008**,768-775.
[19] Liang M.T, Davies N.M, Toth I, *International J. of Pharmaceutics*.**2005**, 301, 247–254.
[20] Wenli L, Guo J, Jin Li, Wang X, Jianying Li, Ping Q,*Drug Development and Industrial Pharmacy*.**2006**, 32(3), pp 309-314.