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Formulation, and evaluation of ketoconazole liposomal drug delivery system

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ABSTRACT

The drug release from Liposomes depends on many factors including the composition of Liposomes, the type of drug encapsulated and nature of the cell. Once it is released a drug that normally crosses the membrane of a cell will enter the cell, other drugs will not enter. This study aimed at developing and optimizing liposomal formulation of Ketoconazole in order to improve its bioavailability. In evaluation study the effect of the varying composition of lipids on the properties such as encapsulation efficiency, particle size and drug release were studied. Phase transition study was carried out to confirm the complete interaction of Ketoconazole with bilayer structure of liposome. Moreover, the release of the drug was also modified and extended over a period of 8 hr in all formulations. F1 emerged as the most satisfactory formulation in so far as its properties were concerned. Further, release of the drug from the most satisfactory formulation (F1) was evaluated through dialysis membrane to get the idea of drug release.

Keywords: Liposomes, Ketoconazole, bioavailability, thin film hydration technique, in vitro drug release studies.

INTRODUCTION

Many of the drug substances are characterized by poor aqueous solubility, which causes many formulation problems. Besides the use of co-solvents, drug complexation and solubilization in surfactant micelles, incorporation in colloidal carrier systems represents an alternative way to render poorly water soluble drugs applicable for effective therapy. Furthermore incorporation of drugs in particulate carriers provides a possibility to manipulate the drug release1. In last few years the colloidal carriers have been used for site specific targeting especially in cancer chemotherapy Based on the carrier material the conventional vehicles used as drug carriers can be divided into 2 groups

1. Polymeric carriers
2. Lipidic carriers:

a. Liposomes
b. Lipoproteins
c. Lipid O/W emulsions
d. Lipospheres

The lipidic carriers are more preferred than polymeric carriers to avoid potential toxicological problems[2]. The vehicles of all the above lipidic carriers are composed of physiological lipids such as phospholipids, cholesterol, cholesterol esters and triglycerides.

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Liposomes[3,4]

Amphiphilic molecules such as phospholipids can be used to form hydrophobic and hydrophilic compartments with in an aqueous environment. Phospholipids have a pair of long hydrocarbon chain(s) covalently bonded to a polar, zwitter ionic and/or ionic head group. Upon dispersion in aqueous medium, they can assume three possible forms Bangham and colleagues first discovered "Liposomes" in the early 1960's subsequently it became the most explored drug delivery systems. It took several years from early to late 60's before the system was realized as a potential drug carrier in the last 15 years5. Liposomes were used to describe one or more concentric lipid bilayers enclosing an equal number of aqueous compartments. Liposomes have been used as drug carriers for several drugs to reduce toxicity or to deliver the drug at the site of infection and have now been formulated into various dosage forms[6]."Structurally liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecule that are usually phospholipids". For the treatment of the following systemic fungal infections: candidiasis, chronic mucocutaneous candidiasis[7] The present study involves preparation and evaluation of liposome containing KetoconazoleLiposomes are prepared by the thin film hydration method using the Phosphotidyl choline as the phospholipid. prepared liposomes were characterized by scanning electron microscopic method respectively.The In-vitro release studies were performed.The prepared liposomes were evaluated for size, shape, drug entrapment efficiency, In-vitro drug release and stability[8]. Ketoconazole interacts with 14-α demethylase, a cytochrome P-450 enzyme necessary for the conversion of lanosterol to ergosterol. This results in inhibition of ergosterol synthesis and increased fungal cellular permeability. Other mechanisms may involve the inhibition of endogenous respiration, interaction with membrane phospholipids, inhibition of yeast transformation to mycelial forms, inhibition of purine uptake, and impairment of triglyceride and/or phospholipid biosynthesis[9]. Ketoconazole can also inhibit the synthesis of thromboxane and sterols such as aldosterone, cortisol, and testosterone.

MATERIALS AND METHOD

Materials

Ketaconazole was collected as a gift sample from Aurobindo Laboratories Ltd, Hyd , polymers and other excipients were purchased from A.R chemicals Pvt.Ltd, HYD

Methodology

Drug excipient compatibility studies[10]

Drug excipients compatibility studies were performed to know the compatibility of excipient with drug at accelerated conditions. The study was conducted by preparing homogenous mixture of excipients with drug and filled in high density polyethylene bags and low density poly ethylene bags.Glass vials were exposed to 60^0C and 40^0C/75 % relative humidity for 4 weeks and low density polyethylene bags were exposed to 40^0C±75 % relative humidity for 4 weeks. Samples were observed periodically for any physical change.

Preparation Of Liposomes

Formulation Table-1

Formulation no	ketaconazole	Cholesterol	Phosphatidylcholine
F1	20	100	100
F2	20	100	200
F3	20	100	300
F4	20	100	400

Method

Liposomes were prepared by thin film hydration technique by using rotary evaporator and using different ratio of lipids. In this method the lipids were dissolved in chloroform. This solution of lipids in chloroform was spread over flat bottom conical flask. The solution was then evaporated at room temperature without disturbing the solution. The hydration of lipid film form was carried out with aqueous medium phosphate buffer (pH 7.4). For this the flask was inclined to one side and aqueous medium containing drug to be entrapped was introduced down the side of flask and flask was slowly returned to upright orientation. The fluid was allowed to run gently over lipid layer and flask was allowed to stand for 2 h at 37^0C for complete swelling. After swelling, vesicles are harvested by swirling the contents of flask to yield milky white suspension. Then formulations were subjected to centrifugation. Different batches of liposomes were prepared in order to select an optimum formula. All batches of liposomes were prepared as per the general method described above.

Evaluations of liposomes

Drug entrapment efficiency of liposomes[11]

Entrapment efficiency of liposomes were determined by centrifugation method. Aliquots (1 ml) of liposomal dispersion were subjected to centrifugation on a laboratory centrifuge (Remi R4C) at 3200 rpm for a period of 90 min. The clear supernatants were removed carefully to separate non entrapped ketaconazole and absorbance recorded. The sediment in the centrifugation tube was diluted to 100 ml with phosphate buffer pH 7.4 and the absorbance of this solution was recorded. Amount of ketaconazole in supernatant and sediment gave a total amount of ketaconazole in 1 ml dispersion. % entrapment of drug was calculated by the following formula

$$\textbf{\% Drug Entrapped (PDE)} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \text{ X } 100$$

Particle size analysis[12]

The particle size of liposomes was determined by using motic digital microscope model No. DMW. All the prepared batches of liposomes were viewed under microscope to study their size. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles were determined.

In Vitro Drug release study[13]

The release studies were carried out in 10 ml franz diffusion cell containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (10ml) was placed in a 10 ml franz diffusion cell . The franz diffusion cell was assembled on a magnetic stirrer and the medium was equilibrated at 37 ± 5^0C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non entrapped ketaconazole liposomal dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Stability Studies[14]

The formulations were subjected to stability studies by storing at 4^0C, 25^0C, 37^0C for 30 days and were analyzed for its % drug entrapped at an interval of 30 days. Stability studies were carried out for a period of two month at 4 ± 2^0C, 25 ± 2^0C and 37 ± 2^0C. The entrapment efficiency was estimated at an interval of 90 days.

RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)

The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipidmixture,which confirmed the absence of any chemical inter action between the drug, lipid and other chemicals.

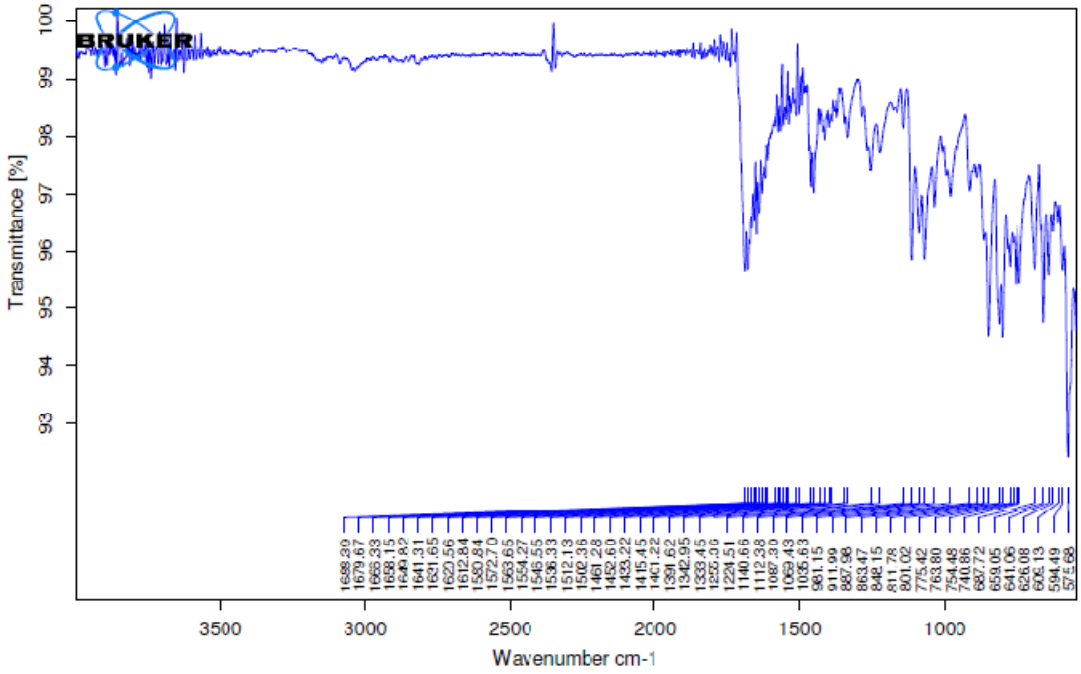

Fig :1 FTIR Studies of Pure Drug

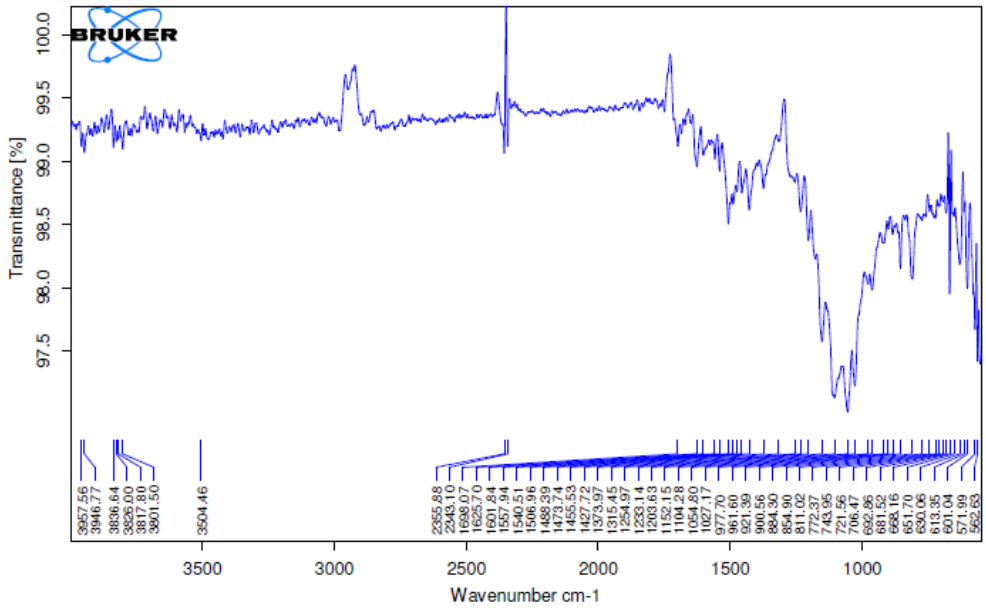

Fig 2 FTIR Studies of Optimization

Particle size

Vesicle shape

Vesicle shape of the prepared formulation was found to be spherical from the SEM(scanning electron microscope) analysis at 15.00kV

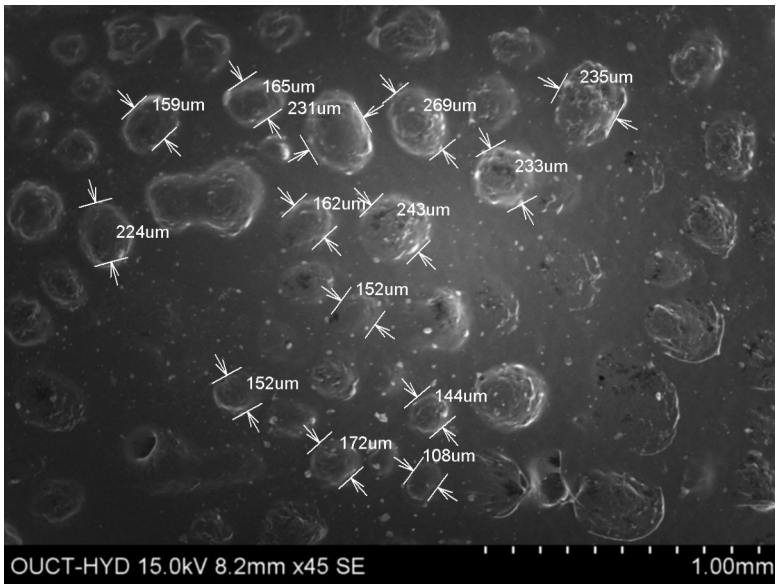

Fig: 3 Particle size of optimization formulation

Table: 2 Mean particle size (mps) of different formulation of liposomes

Sr. No	Formulation No.	Particle size(µm)
1	F1	234
2	F2	213
3	F3	218
4	F4	210

Drug entrapment efficiency

Table :3 Different batches of liposome made by using different ratio of lipids

Sr. No	Formulation no.	PDE
1	F1	91.25
2	F2	90.31
3	F3	75.74

4	F4	63.22

Drug release studies

Table :4 Cumulative percentage drug release from various formulation of liposomes

Time	Batch code			
	F1	F2	F3	F4
0	0	0	0	0
1	18.95	16.50	13.12	15.25
2	22.65	21.18	24.21	22.10
3	37.21	38.30	32.28	34.26
4	45.31	44.35	41.50	40.52
5	61.52	59.24	52.25	52.85
6	74.32	71.60	66.54	63.48
7	86.21	83.28	71.08	77.20
8	93.28	91.10	84.95	84.50

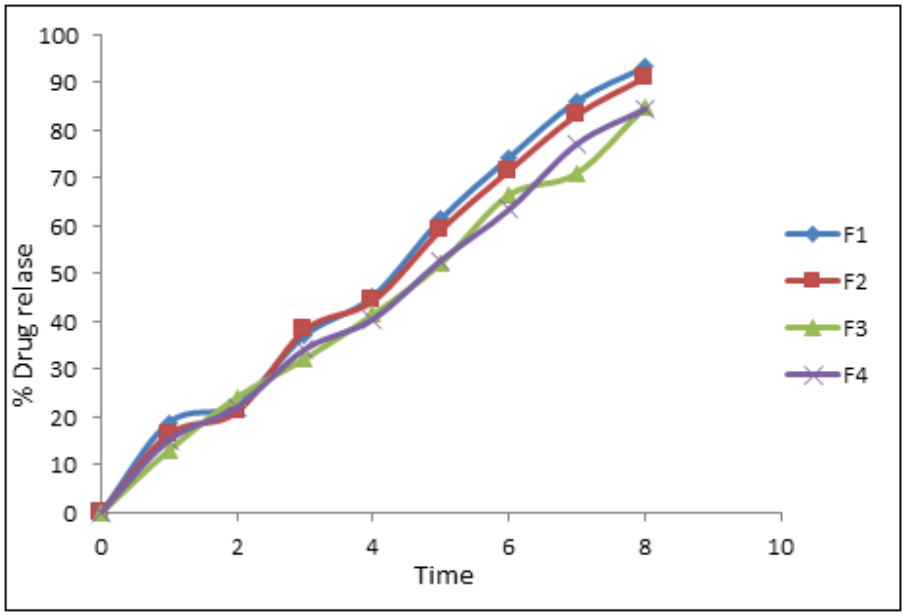

Fig :4 *In vitro* drug release of various formulations

All the three batches of formulation F1 were found to release the drug in 8 h. The cumulative percentage release was found to be 93.28 %.

Stability studies

There was no significant change in physical and chemical properties of the tablets of formulation F-1 after 3 months. Parameters quantified at various time intervals were shown;

Table- : 5 Results of stability studies of optimized formulation F-1

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-1	**25^0C/60%RH % Release**	93.28	93.20	93.17	93.11	**Not less than 85 %**
F-1	**30^0C/75% RH % Release**	93. 28	93.21	93.18	93.12	**Not less than 85 %**
F-1	**40^0C/75% RH % Release**	93. 28	93.18	93.11	93.08	**Not less than 85 %**

CONCLUSION

From the performed work it was concluded that:ketaconazole possesses all requisite qualities required for liposomal drug delivery. Among the various formulation, the combination F1 was found to be most suitable because of high encapsulation efficiency with smaller particle size.The formulation F1 comprising phosphatidylcholine, cholesterol, fulfills the requirement of good liposomal formulation. In vitro drug release up to 8 hrs and more than 93.28% drug released. It shows encapsulation efficiency of 91.25 % and particle size of 234µm.

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