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Research Article

Formulation and Evaluation of Liposomes of Ketoconazole

Dr. Rakesh P. Patel[1*], Hardik Patel[1] and Ashok H. Baria[2]

[1]Department of Pharmaceutics, S. K. Patel College of Pharmaceutical Education and Research, Ganpat Univesity, Kherva. Mehsana, Gujarat, India, 382711
[2]S J Thakkar Pharmacy College, Rajkot, Gujarat, India.

ABSTRACT
Antifungal drug, Ketoconazole was encapsulated in liposomes for topical application. Ketoconazole liposomes were prepared by thin film hydration technique using soya lecithin, cholesterol and drug in different weight ratios. The prepared liposomes were characterized for size, shape, entrapment efficiency, *in-vitro* drug release (by franz diffusion cell) and physical stability. The studies demonstrated successful preparation of Ketoconazole liposomes and effect of soya lecithin: cholesterol weight ratio on entrapment efficiency and on drug release.

Keywords: antifungal, liposomes, ketoconazole, soya lecithin, cholesterol

INTRODUCTION

A tremendous amount of work has been done to formulate drugs in sustained and controlled release dosage forms for oral and parenteral administration. To pursue optimal drug action, functional molecules could be transported by a carrier to the site of action and released to perform their task, for which the carrier itself should be non toxic, biodegradable, and of suitable shape and size to accommodate wide variety of substances. Liposomes are microscopic lamellar structures formed on the admixture of soya lecithin, cholesterol and tocopheryl acetate with subsequent hydration in aqueous media. Liposomes have been widely evaluated for controlled and targeted drug delivery for treatment 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 that they may act as slow releasing vehicles.

Ketoconazole (KTZ) is a broad spectrum antifungal agent active against a wide variety of fungi and yeasts. It is readily but incompletely absorbed after oral dosing and is highly variable. Topically it is used in the treatment of candidal or tinea infections of the skin. Encapsulation of KTZ in liposomes may increase the half life providing prolonged drug delivery and minimize the commonly occurring side effects.

The objective of the present work is to prepare KTZ liposome and study the *in -vitro* drug release and stability studies of prepared liposomes.

*Corresponding author:
Dr. Rakesh P. Patel,
Asst. Professor and Head of Pharmaceutics & Pharma. Technology Department,S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva. PIN: 382711.
City: Mehsasan, State: Gujarat, Country: India
E-mail: raka_77us@yahoo.com

MATERIALS AND METHOD

Materials

KTZ was received as a gift sample from Yash Pharmaceutical Ltd, Himmatnagar. Soya lecithin (HiMedia Laboratories Pvt. Ltd., Mumbai), cholesterol (S.D. Fine Chem. Ltd., Mumbai), Tocopheryl acetate (Morvel Laboratories Pvt. Limited, Mehsana) and cellulose acetate membrane (Sartorious cellulose acetate membrane) were used in the study. All other chemicals and solvents were of analytical or pharmacopoeial grade.

Preparation

Multilamellar vesicles of KTZ were prepare by thin film hydration technique using rotary flash evaporator [1,2]. The liposomes were prepared using Soya lecithin (neutral charge), cholesterol (neutral charge) and tocopheryl acetate by thin film hydration technique. The effect of various process variables such as speed of rotation, vacuum, temperature and hydration time was altered and the effect on the formation of uniform thin lipid film was evaluated. Here Drug:Soya lecithin:Cholestrol ratios, volume of organic phase and volume aqueous phase were altered and drug entrapment efficiency was studied [2,3].

Drug:Soya lecithin:Cholestrol in the ratio 2:10:1(weight ratio) and tocopheryl acetate (equivalent to 1% w/w of lecithin taken) were dissolved in 14 ml dichloromethane [4-6]. This solution was taken in a 250 ml round bottom flask. The flask was rotated in rotary flash evaporator at 80 rpm for 15 minutes in thermostatically controlled water bath 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 5 ml

of distilled water [7]. The flask was once again rotated at the same speed as before and at room temperature for 1 hr [8]. The liposomal suspension was left to mature overnight at 4ºC, to ensure full lipid hydration [9].

The unentrapped drug was removed from liposomal suspension by centrifugation at 5000 rpm for 15 minutes at 4°C temperature by using remi cooling centrifuge. 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. Pellets were resuspended in distilled water [9,10].

EVALUATION

Liposomal formulations after their formulation and processing for a specified purpose are characterized to ensure their predictable *in-vitro* and *in-vivo* performances. The liposomes produced by different techniques may have different physicochemical characteristics. These differences may have an impact on their behaviour *in-vivo* (disposition) and *in-vitro*. There are several examples demonstrating the importance of proper selection of liposomes structure to optimize therapeutic effect. The characterization parameters for the purpose of evaluation could be classified into three broad categories, which include physical, chemical and biological parameters. Physical characterization evaluates various parameters, including size, shape, surface features, lamellarity, phase behaviour and drug release profile. Chemical characterization includes those studies which establish the purity and potency of various liposomal constituents. Biological characterization parameters are helpful in establishing the safety and suitability of the formulations for the in vivo use or the therapeutic application. Physical and chemical characterizations are very important for meaningful comparison of different liposomes preparations for different batches. Biological consideration helps to ensure safety of use in humans [11-13].

Combination of various characterization methods are used to characterize liposomes. Liposomes characterization should be performed immediately after preparation. One should also ensure that no major changes occur on storage so that well characterized product is supplied which should show optimal and reproducible clinical effects. Some of the parameters characterized in liposome product development are size and size distribution, surface topology, encapsulation efficiency, capture volume, lamellarity and *in-vitro* drug release profile [14,15].

Liposomes containing KTZ, prepared by thin film hydration technique, were characterized for following attributes.

Optimization of formulation using 3^2 full factorial design

It is desirable to develop an acceptable pharmaceutical formulation in shortest possible time using minimum number of man-hours and raw materials. Traditionally pharmaceutical formulations after developed by changing one variable at a time by trial and error method which is time consuming in nature and requires a lot of imaginative efforts. Moreover, it may be difficult to develop an ideal formulation using this classical technique since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial design. In addition to the art of formulation, the technique of factorial design is an effective method of indicating the relative significance of a number of variables and their interactions.

The number of experiments required for these studies is dependent on the number of independent variables selected. The response (Y_i) is measured for each trial.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Where Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs and b_i is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1X_1 and X_2X_2) are included to investigate non linearity. The polynomial equation can be used to draw conclusion after considering the magnitude of coefficient and the mathematical sign it carries (i.e. positive or negative).

A 3^2 randomized full factorial design was utilized in the present study. In this design two factors were evaluated, each at three levels, and experimental trials were carried out at all nine possible combinations. The design layout and coded value of independent factor is shown in Table 1. The factors were selected based on preliminary study. The weight ratio of drug : soya lecithin : cholesterol and volume of hydration medium were selected as independent variables. The % Entrapment efficiency was selected as dependent variable [16,5]. The formulations of the factorial batches (F_1 to F_9) are shown in Table 1.

Table 1: 3^2 Full factorial design layout

Batch code	X_1	X_2
F_1	-1	-1
F_2	-1	0
F_3	-1	1
F_4	0	-1
F_5	0	0
F_6	0	1
F_7	1	-1
F_8	1	0
F_9	1	1

Coded value	Drug : PC : Cholesterol weight ratio* X_1	Hydration Volume (ml) X_2
-1	1.5 :10 : 1	4
0	2 :10 : 1	5
1	2.5 :10 : 1	6

* Where, 1 = 20

Microscopy

Morphology of liposomes was studied under microscope. All batches of the liposomes prepared were

viewed under Leica DMIL inverted fluorescence microscope to study their shape and lamellarity. The liposomal dispersion was suitably diluted on glass slide and viewed by microscope with magnification of 45 x [9, 10, 3].

Particle size and particle size distribution

The mean particle size and particle size distribution of the optimized batch was obtained by particle size analyzer (Sympatec HELOS, Germany (H1004)). The instrument measures the particle size based on the laser diffraction theory. The apparatus consists of a He-Ne laser beam of 632.8 nm focused with a minimum power of 5 mW using a fourier lens [R-5] to a point at the center of multielement detector and a sample holding unit (Su cell). The sample was stirred using a stirrer before determining the vesicle size. The vesicle dispersions were diluted about 100 times in the deionized water. Diluted liposomal suspension was added to sample dispersion unit containing stirrer and stirred at high speed in order to reduce interparticles aggregation and laser beam was focused [16].

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 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 222 nm [17, 18, 9]. The entrapment efficiency was then calculated using following equation.

$$\%\ Entrapment\ efficiency = \frac{\text{Entrapped drug}}{\text{Total drug added}} \times 100$$

D ifferential scanning calorimetry (DSC) study

Differential scanning calorimetry (DSC) experiments were performed with differential scanning calorimeter (model TA-60, Shimadzu, Japan). Samples of pure KTZ, soya lecithin, cholesterol and drug-loaded multilamellar liposomes were submitted to DSC analysis. The analyses were performed on 5 mg samples sealed in standard aluminum pans. Thermograms were obtained at a scanning rate of 20°C/min. Each sample was scanned between 0°C to 200°C. The temperature of maximal excess heat capacity was defined as the phase transition temperature [9, 19].

***In-vitro* drug release study from liposomes**

Studies of the drug release/diffusion from liposomal system are directed toward the approaches that are relevant to the in vivo condition. *In vitro* diffusion studies were carried out using Franz diffusion cell. Apparatus with a diameter of 25 mm and a diffusional area of 4.90 cm^2. Regenerated cellulose acetate membrane (thickness of 60–65 µm and 0.45 µm pore size) was sandwiched between the lower cell reservoir and the glass cell top containing the sample and secured in place with a pinch clamp. The receiving compartment (volume 22 ml) was filled with pH 5 acetate buffer containing 20% v/v methanol (to maintain sink condition) [20, 21]. The system was maintained at 37±0.5 °C by magnetic heater, resulting in a membrane-surface temperature of 32 °C [22]. A TeflonTM coated magnetic bar continuously stirred the receiving medium to avoid diffusion layer effects. A sample was placed evenly on the surface of the membrane in the donor compartment. 2 ml of receptor fluid were withdrawn from the receiving compartment at 1, 2, 3, 4, 6, 8, 10 & 12 hours and replaced with 2 ml of fresh solution. Samples were assayed spectrophotometrically for drug content at 222 nm. [23].

Storage stability of liposomes

Physical stability study was performed to investigate the leak out of the drug from liposomes during storage. Liposomal suspensions of KTZ of optimized batch (F_4) were sealed in 20-ml glass vials and stored at refrigeration temperature (2-8°C), room temperature (25±2°C) and 45°C for a period of 1 month. Samples from each liposomal formulation were withdrawn at definite time intervals. The residual amount of the drug in the vesicles was determined after separation from unentrapped drug as described previously under the section drug entrapment efficiency [24,3,9].

RESULT AND DISCUSSION

Influence of formulation variables on characteristics of liposomes

Influence of the composition of liposomes of KTZ on the entrapment efficiency was studied by preliminary experiments and 3^2 full factorial designs.

Table 2: Responses of 3^2 factorial design batches

Sr. No.	Batch code	% Entrapment efficiency*
1	F_1	38.56 ± 0.53
2	F_2	34.76 ± 0.96
3	F_3	28.84 ± 0.62
4	F_4	54.41 ± 0.19
5	F_5	48.18 ± 0.24
6	F_6	41.85 ± 0.36
7	F_7	47.66 ± 0.13
8	F_8	42.21 ± 0.20
9	F_9	34.28 ± 0.24

* Values are represented as mean ± SD (n=3).

From many of different preliminary experimental batches soya lecithin: cholesterol ratio 10:1 and 14 ml volume of organic phase were kept as constant for 3^2 full factorial design batches. While drug : soya lecithin : cholesterol (weight ratio) and volume of hydration medium were selected as variable factors. According to experimental design nine experiments were conducted as shown in Table 1. The percentage of drug entrapped within liposomes was selected as dependent variable as shown in Table 2 for 3^2 full factorial design. The major process parameters and the formulation parameters were optimized using the percentage of drug entrapment as response parameter by preliminary trials.

Table 3: Full model for % entrapment efficiency

Parameter	Co-efficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	48.67	0.41	119.73	0.000001	47.38	49.96	47.38	49.96
X_1	3.66	0.22	16.46	0.000480	2.96	4.37	2.96	4.37
X_2	-5.94	0.22	-26.68	0.000110	-6.65	-5.23	-6.65	-5.23
X_1*X_2	-0.92	0.27	-3.36	0.043880	-1.78	-0.05	-1.78	-0.05
X_1^2	-10.43	0.39	-27.04	0.000110	-11.66	-9.20	-11.66	-9.20
X_2^2	-0.78	0.39	-2.03	0.135180	-2.01	0.44	-2.01	0.44

Table 4: Summary output of % entrapment efficiency

Regression Statistics	Multiple R	R Square	Adjusted R Square	Standard Error	Observations
	0.999134	0.998268	0.995382	0.545384	9

Table 5: ANOVA for % entrapment efficiency

	df	SS	MS	F	Significance F
Regression	5	514.401693	102.880339	345.882290	0.000244
Residual	3	0.892330	0.297443		
Total	8	515.294022			

In 3^2 full factorial design, three levels of each factor were taken as high, medium and low levels with two variables drug : soya lecithin : cholesterol (weight ratio) and volume of hydration medium were taken in this design.

The percentage entrapment efficiency of different liposomal batches of 3^2 full factorial design was found to be between ranges 28.84±0.62 % to 54.41±0.19 %. The maximum entrapment was observed in batch F_4 i.e. 54.41±0.19 %.

Data were analyzed statistically by one-way analysis of variance (ANOVA) using Microsoft Excel 2003 and by the student's *t*-test (level of significance for $p<0.05$).

Factorial equation for percentage entrapment efficiency

The polynomial equation was generated by multiple linear regressions. The equation derived is as under :

$$Y = 48.66 + 3.66X_1 - 5.94X_2 - 0.91X_1X_2 - 10.42X_1^2 - 0.78X_2^2$$

The data clearly indicate that the % entrapment efficiency values are strongly dependent on the selected independent variables. The fitted equation (for full model) relating the response (% entrapment efficiency) to the transformed factor is shown by equation 3. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e. positive or negative). Table 5 shows the results of the analysis of variance (ANOVA) which was performed to identify insignificant factors.

The high values of correlation coefficient for % entrapment efficiency (Table 4) indicate a good fit. The equations may be used to obtain estimates of the response as a small error of variance was noticed in the replicates. The results of statistical analysis are shown in Table 3. The coefficients b_1, b_2, b_{12} and b_{11} were found to be significant at $P<0.05$. While the significance level of coefficient b_{22} was found to be $P = 0.1351$ which is >0.05 so it may be concluded that interaction term b_{22} does not contribute significantly to the prediction of % entrapment efficiency.

The results of regression analysis reveal that, on increasing the values for X_1, increase in % entrapment efficiency is observed, because coefficient b_1 bears a positive sign. While on increasing the values for X_2, decrease in % entrapment efficiency is observed, because coefficient b_2 bears a negative sign.

From the above study, it was found that the entrapment of KTZ in liposomes primarily depends on the lipid composition, drug to lipid ratio and also the volume of hydration medium. The requirement of mechanical stability and rigidity was fulfilled by incorporating cholesterol, which is well documented as being to

a) Decrease the fluidity or micro viscosity of the bilayers by filling empty spaces among the phospholipids molecules, anchoring them more strongly into the structure.
b) Reduced the permeability of the membrane to water soluble molecules due to above effect.
c) Stabilize the membrane in the presence of biological fluids such as plasma.

From the 3^2 full factorial design, it had been observed that at the 2:10:1 drug : soya lecithin : cholesterol weight ratio and 4 ml hydration volume maximum entrapment was obtained. While by changing these drug: soya lecithin : cholesterol weight ratio and volume of hydration entrapment was found to be decreased.

Microscopy

Morphology of liposomes was studied under microscope. All batches of the liposomes prepared were viewed under Leica DMIL inverted fluorescence microscope to study their shape and lamellarity. The liposomal dispersion was suitably diluted on glass slide and viewed by microscope with magnification of 45 x.

Table 6: Particle size distribution of optimized batch (F_4) by Sympatec particle size Analyzer

Volume Size Distribution

x0/µm	Q3/%	x0/µm	Q3/%	x0/µm	Q3/%	x0/µm	Q3/%
0.18	0.01	0.74	25.20	3.00	88.88	12.20	100.00
0.22	0.10	0.86	33.91	3.60	91.83	14.60	100.00
0.26	0.37	1.00	43.50	4.20	93.82	17.40	100.00
0.30	0.94	1.20	55.22	5.00	95.62	20.60	100.00
0.36	2.45	1.50	68.04	6.00	97.06	24.60	100.00
0.44	5.63	1.80	75.95	7.20	98.18	29.40	100.00
0.52	10.00	2.10	80.88	8.60	99.05	35.00	100.00
0.62	16.56	2.50	85.23	10.20	99.66		

x10 =	0.52 µm	x50 =	1.11 µm	x90 =	3.23 µm
x16 =	0.61 µm	x84 =	2.39 µm	x99 =	8.52 µm
VMD =	1.6 µm	c_opt =	4.24 %		

Figure 1 (A, B, C) shows photomicrograph of liposomal formulation of optimized batch F_4 prepared by thin film hydration technique by Leica DMIL inverted fluorescence microscope under 45x magnification. The photomicrograph of optimized batch F_4 shown in Figure 1 also reveals the presence of well-identified spheres of multilamellar vesicles that consist of many concentric phospholipids bilayers. Thus, formation of multilamellar vesicles was confirmed by examining the liposomal suspension under microscope with the magnification power of 45x.

Table 7: Cumulative percentage drug release from factorial design batches after 12 hours

Sr. No.	Batch Code	Cumulative percentage drug release after 12 hours*
1	F_1	42.17 ± 0.51
2	F_2	45.17 ± 0.40
3	F_3	49.20 ± 1.22
4	F_4	34.96 ± 0.86
5	F_5	39.87 ± 0.96
6	F_6	43.27 ± 0.63
7	F_7	36.93 ± 0.65
8	F_8	42.13 ± 1.10
9	F_9	45.37 ± 0.61

*Values are represented as mean ± SD (n=3)

Particle size and particle size distribution

The particle size and particle size distribution of liposomes containing KTZ were determined using method described previously. Table 6 shows the particle size distribution of the optimized liposomal batch (F_4) from 3^2 factorial design.

The particle size of optimized liposomal batch was found to be between ranges 0.86 µm to 1.50 µm. Figure 2 shows particle size distribution of optimized liposomal batch (F_4) and the mean particle size of the optimized batch was between 0.86 µm to 1.50 µm.

Drug entrapment efficiency

The entrapment of KTZ in liposomes was determined using the method elaborated in section previously. Table 2 shows the result of these determinations. The percentage entrapment efficiency of different liposomal batches of 3^2 full factorial design was found to be between ranges 28.84±0.62 % to 54.41±0.19%.

The maximum entrapment was observed in batch F_4 i.e. 54.41±0.19 %. It can be concluded that the formulation component variables i.e. drug : soya lecithin : cholesterol ratio, volume of organic phase and volume of aqueous phase and formulation process variables i.e. speed of rotation, vacuum, temperature and hydration time affect the entrapment efficiency of drug.

Differential scanning calorimetry study

DSC study was carried out for KTZ, soya lecithin, cholesterol and drug loaded multilamellar liposomes using the method described in section previously. DSC thermograms of KTZ, soya lecithin, cholesterol and drug loaded multilamellar liposomes are illustrated in figure 3.

DSC thermogram of KTZ showed endotherm at 152.89°C. DSC thermogram of soya lecithin and cholesterol showed endotherm at 128.93°C and 148.27°C, respectively. DSC thermogram of KTZ loaded liposomes composed of soya lecithin : cholesterol (10:1) weight ratio interestingly showed disappearance of the melting endotherm of KTZ and the major endotherm was observed at 137.28°C. The melting endotherm of soya lecithin was found to be shifted from 128.93°C to 137.28°C, signifying that all the lipid components interact with each other to a great extent while forming the lipid bilayer. The incorporated KTZ associated with lipid bilayers and interacted to a large extent with them. Absence of the melting endotherm of KTZ and shifting of the lipid bilayer components endotherm suggested significant interaction of KTZ with bilayers, indicating the interaction of KTZ with bilayers leading to enhanced entrapment of the drug and decreased rate of release. The DSC results of liposomes suggest enhanced entrapment efficiency of KTZ in the lipid bilayer.

Table 8: Full model for % cumulative drug release after 12 hours

Parameter	Co-efficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	39.64	0.43	93.21	0.000003	38.28	40.99	38.28	40.99
X_1	-2.02	0.23	-8.67	0.003230	-2.76	-1.28	-2.76	-1.28
X_2	3.96	0.23	17.02	0.000440	3.22	4.70	3.22	4.70
X_1*X_2	0.35	0.29	1.24	0.304520	-0.56	1.26	-0.56	1.26
X_1^2	4.13	0.40	10.23	0.001980	2.84	5.41	2.84	5.41
X_2^2	-0.41	0.40	-1.01	0.387700	-1.69	0.88	-1.69	0.88

Table 9: Summary out put of cumulative percentage drug release

Regression Statistics	Multiple R	R Square	Adjusted R Square	Standard Error	Observations
	0.996836	0.993683	0.983154	0.570541	9

Table 10: ANOVA for cumulative percentage drug release after 12 hours

	df	SS	MS	F	Significance F
Regression	5	153.604136	30.720827	94.375321	0.001695
Residual	3	0.976553	0.325518	-	-
Total	8	154.580689	-	-	-

The percentage cumulative drug release of different liposomal batches of 3^2 full factorial design was found to be between ranges 34.96 ± 0.86 % to 49.20 ± 1.22 %. The % cumulative drug release in optimized batch F_4 was observed to be 34.96 ± 0.86 %. Figure 4 shows the plot of % cumulative drug release against time (hours) for factorial design batches.

Data were analyzed statistically by one-way analysis of variance (ANOVA) using Microsoft Excel 2003 and by the student's *t*-test (level of significance for $p<0.05$).

Factorial equation for percentage cumulative drug release after 12 hours

The polynomial equation was generated by multiple linear regressions. The equation derived is as under

$$Y = 39.64 - 2.02X_1 + 3.96X_2 + 0.35X_1X_2 + 4.13X_1^2 - 0.41X_2^2$$

The data clearly indicate that the % cumulative drug release values are strongly dependent on the selected independent variables. The fitted equation (for full model) relating the response (% cumulative drug release) to the transformed factor is shown by equation 4. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e. positive or negative). Table 10 shows the results of the analysis of variance (ANOVA) which was performed to identify insignificant factors.

The high values of correlation coefficient for % cumulative drug release (Table 9) indicate a good fit. The equations may be used to obtain estimates of the response as a small error of variance was noticed in the replicates. The results of statistical analysis are shown in Table 8. The coefficients b_1, b_2 and b_{11} were found to be significant at $P<0.05$. While the significance level of coefficient b_{12} and b_{22} were found to be $P = 0.3045$ and $P = 0.3877$ respectively which is >0.05 so it may be concluded that interaction term b_{12} and b_{22} does not contribute significantly to the prediction of % cumulative drug release.

The results of regression analysis reveal that, on increasing the values for X_1, decrease in % cumulative drug release is observed, because coefficient b_1 bears a negative sign. While on increasing the values for X_2, increase in % cumulative drug release is observed, because coefficient b_2 bears a positive sign.

From the above study, it was found that the drug release from liposomes primarily depends on the lipid composition, drug to lipid ratio and also the volume of hydration medium.

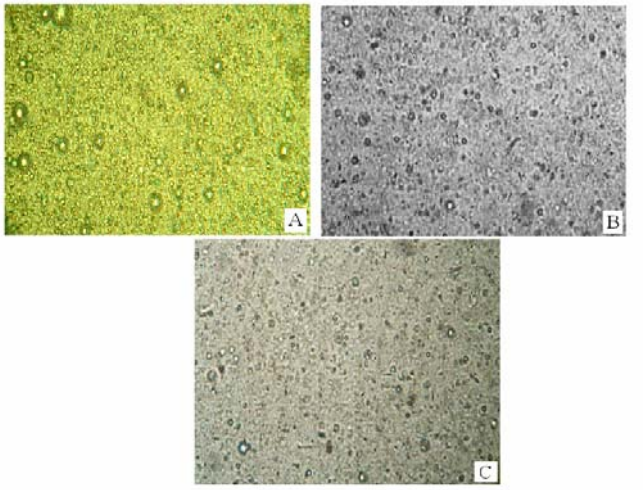

Figure 1: Photomicrograph of liposomal formulation of optimized batch F_4

Storage stability of liposomes

Here, one month stability study of liposomal suspension was conducted with respect to the liposomes' ability to retain an entrapped drug during a defined time period.

The results of stability studies are shown in figure 5 as percentage of drug remaining in liposomes under different

storage conditions, i.e. at refrigeration condition (2-8°C), at room temperature (25±2°C) and at 45°C. Figure 5 shows

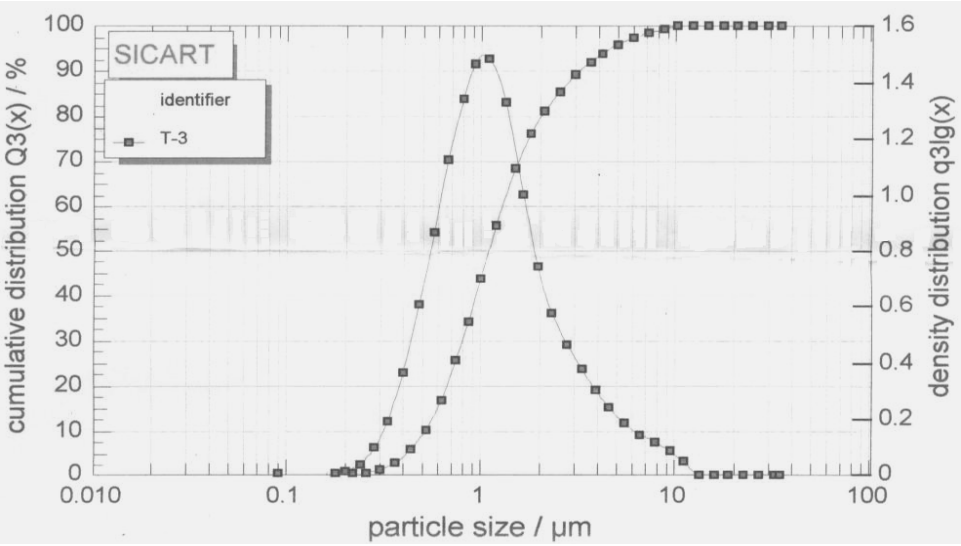

Figure 2: Particle size distribution of optimized batch (F_4) by Sympatec particle size analyzer

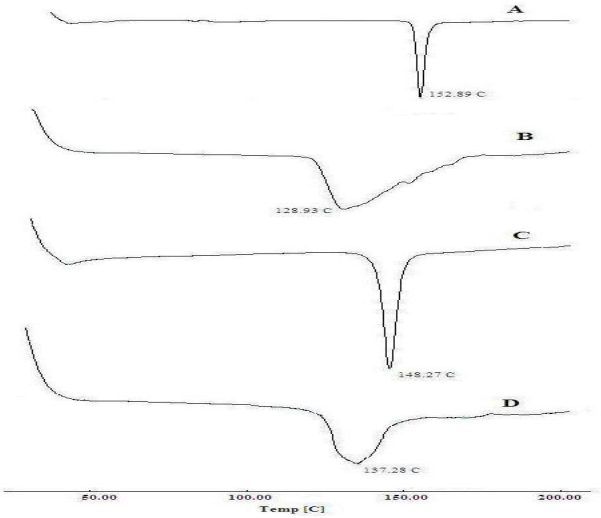

Figure 3: DSC thermograms of KTZ (A), soya lecithin (B), cholesterol (C), and drug loaded multilamellar liposomes (D).

liposomes were relatively stable at refrigerated storage condition. The drug leakage percent amounts of original entrapped in liposomes are very small (< 5%) at 2-8°C and have no significant difference after 1 month compared with immediately after preparation. The results of drug retention studies show higher drug leakage at higher temperature. This may be due to the higher fluidity of lipid bilayers at higher temperature, resulting in higher drug leakage. Loss of drug from the vesicles stored at elevated temperatures may be attributed to the effect of temperature on the gel to liquid transition of lipid bilayers together with possible chemical degradation of the phospholipids, leading to defects in membrane packing. Acceleration in drug leakage at higher temperatures, as observed in storage stability studies, suggested keeping the liposomal product in the refrigeration condition.

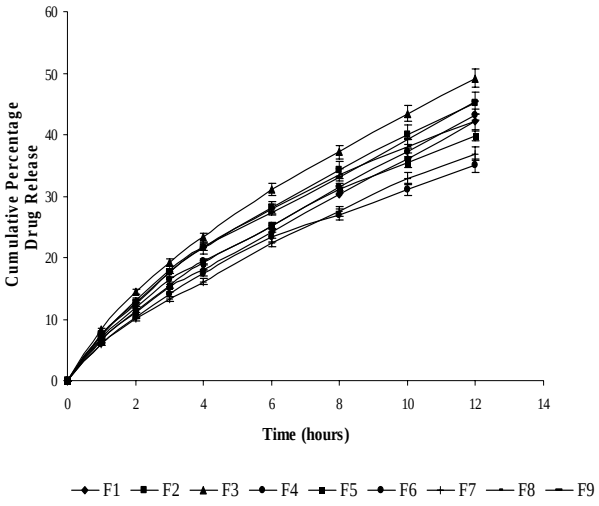

Figure 4: Comparative diffusion studies of factorial design batches. All values are shown as mean ± SD (n=3).

CONCLUSION

The liposomal product of ketoconazole was prepared with the view to improve therapeutic response and reduce the possible adverse symptoms. Here liposomes of ketoconazole were prepared using thin film hydration technique. Percentage entrapment efficiency was optimized after studying the effect of various process and formulation variables. Maximum entrapment efficiency was found to be 54.41±0.19 %. The

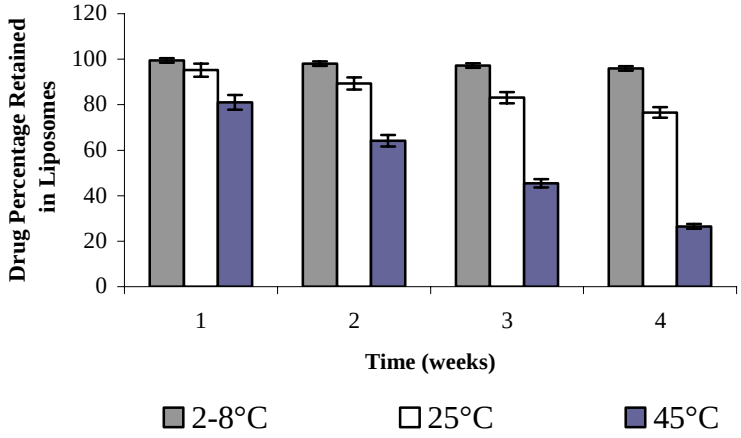

Figure 5: Stability studies-Percentage of drug remaining in liposomes under different storage conditions, at 2-8°C, 25°C and 45°C. All values are shown as mean ± SD (n=3).

prepared liposomes were found to have good morphological properties and size distribution. From DSC thermograms of

liposomes it can be concluded that significant interaction occur between drug and lipid components of the vesicles that lead to higher entrapment efficiency. The percentage cumulative drug release from the optimized batch i.e. F_4 was found to be 34.96±0.86 % after 12 hours of diffusion studies. Stability studies showed maximum percent drug retention at refrigerated temperature (2-8°C). The drug entrapment efficiency can be attributed to phospholipids' ability to vesiculate independently because they carry two bulky nonpolar lipid chains and a polar head group, which helps them spontaneously form into closed bilayer systems.

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