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COMPARATIVE STUDY OF KETOCONAZOLE LIPOSOMES PREPARED WITH COMMERCIAL SOYA LECITHIN AND ENRICHED SOYA LECITHIN

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ABSTRACT

Liposomes have appealing biological properties, including the biocompatibility and biodegradability. They are ensured as active vectors because of their ability to enhance the encapsulant performance by expanding drug solubility and stability, conveying encapsulated medications to specific target sites and showing sustained drug release. The objective of the present study is to develop ketoconazole liposomes with commercial Soya lecithin and enriched Soya Lecithin and make a comparative study. Liposomes were prepared by film hydration technique. The prepared liposomes were evaluated for optical microscopy, entrapment efficacy, In Vitro release studies, Scanning Electron Microscopy and FTIR-Spectroscopy. Encapsulation efficiency of liposomes showed good amount of loading into the vesicles among all liposomal formulations E-F4 showed highest encapsulation efficiency 92.36 %. FTIR study proves that there is no interaction between pure drug and excipients used in the formulation. SEM images shows the surface of the vesicles was smooth and round and size of E-F4 particles are in the range of 149µm - 306µm & for E-F5 the size of the particles are in the range of 136-265µm. In-vitro diffusion studies were performed for liposomes, the results showed that highest percentage 87.39 % of drug release in 24 hrs was shown in for liposome formulation E-F4.

***Key Words:** Liposomes, Ketoconazole, Enriched lecithin, film hydration technique, comparative study.*

INTRODUCTION

In Liposomes membrane can be composed of natural or synthetic lipids which are relatively biocompatible and biodegradable. Liposomes are bilayered due to which they can be used as carriers for both lipophilic and water-soluble molecules [1]. They

are artificial in nature which is prepared with cholesterol and phospholipids. Liposomes are considered to be promising systems in drug delivery due to their size, hydrophilic and hydrophobic nature [2].

Their structure turns liposomes into ideal drug carriers, where hydrophilic drug gets entrapped in the core while the hydrophobic drug will be entrapped in the lipid bilayers. The encapsulation depends on the partition coefficient or LogP value of the drug [3]. Liposomes are widely used as topical agents for treatment of dermatological diseases. Drugs encapsulated in liposomes show enhanced skin penetration [4].

Ketoconazole is a synthetic imidazole antifungal drug used mostly to deal with fungal infections [8]. Ketoconazole comes commercially as a tablet for oral administration in addition to a variety of formulations for topical administration, such as creams (used to treat tinea; cutaneous candidiasis, including candidal paronychia; and pityriasis versicolor) and shampoos, used mostly to deal with dandruff--seborrhoeic dermatitis of the scalp.

Soyabean lecithin is a mixture containing phospholipids, triglycerides, and lesser amounts of other constituents like phytoglycolipids, phytosterols, tocopherols, and fatty acids. Lecithin has potential as a multifunctional additive for food, pharmaceutical, and industrial applications [5]. The phospholipid content of commercial soybean lecithin varies depending on the processing conditions of degumming of soyabean oil [6]. In the present study a simple method was developed to obtain enriched phospholipid content to a required percentage in commercial soyabean lecithin.

MATERIALS AND METHODS

Experimental Work

Materials

Ketoconazole obtained from Strides arco labs, Bengaluru, Soya Lecithin obtained from Sigma chemicals limited, Hyderabad , Cholesterol Finar chemicals, Ahmedabad, Chloroform obtained from molychem, Methanol & Potassium dihydrogen orthophosphate obtained from S.D.Fine chemicals, Sodium hydroxide obtained from qualigens, acetone.

Methods

Preparation of calibration curve

By using absorption maxima a standard curve was plotted within the concentration range of 5- 25µg/ml. The optical density values of resulting solution were measured at 270nm.

Preparation of Enriched Lecithin from Commercial Soya Lecithin

50g of Commercial soybean lecithin was dissolved in 20ml of acetone and the preparation is slowly added to 230ml of chilled acetone. The contents were refrigerated for 60 min at 4.5°C and centrifuged. The acetone layer which is containing neutral lipids was decanted and the insoluble material was extracted with 100ml of chilled acetone followed by centrifugation [6].

Preparation of liposomes

Liposomes were prepared by dispersion method at different ratios of soya lecithin and cholesterol. In this method soya lecithin and cholesterol were dissolved in chloroform.

The lipid solution in chloroform was spread in the flat bottom conical flask. The solution gets evaporated at room temperature. The lipid layer hydration was carried out with aqueous medium phosphate buffer (pH 7.4). Phosphate buffer (pH 7.4) was introduced into the flask containing the lipid layer and flask was allowed to stand for 2 hrs for swelling. After swelling, harvested vesicles were swirled where milky white suspension was obtained. This solution was stored in the refrigerator.

Liposomes were prepared with different compositions in order to select an optimum formula. All compositions of liposomes were prepared as per the (Table 1) by using both Commercial Lecithin and Enriched Lecithin.

Table 1. Composition of Liposomal Formulations

S.No	Formulation	Soya Lecithin	Cholesterol	Drug	Chloroform	Buffer (pH 7.4)
1	F1	240 mg	60 mg	200 mg	5 ml	10 ml
2	F2	210 mg	90 mg	200 mg	5 ml	10 ml
3	F3	180 mg	120 mg	200 mg	5 ml	10 ml
4	E-F4	240 mg	60 mg	200 mg	5 ml	10 ml
5	E-F5	210 mg	90 mg	200 mg	5 ml	10 ml
6	E-F6	180 mg	120 mg	200 mg	5 ml	10 ml
Note: *E - indicates liposomes prepared from enriched lecithin						

Characterization of liposomes

Optical microscopy

The prepared ketoconazole liposomes were viewed under microscopy for observing the vesicle formation and discreteness of dispersed vesicles. A slide was prepared by placing a drop of liposome dispersion on a glass slide and cover slip was placed over it and this slide was viewed under optical microscope at 40X magnification. Photographs were taken for the prepared slides using digital camera.

Scanning electron microscopy and Size distribution Analysis (SEM)

Particle size of liposomes is a vital factor. The surface morphology and size distribution of liposomes were observed by SEM. A double-sided tape was affixed on aluminum stubs and the liposomal powder was spread on it. The aluminum stub was kept in a vacuum chamber of scanning electron microscope (XL 30 ESEM with EDAX, Philips, Netherlands). The morphological characterization of the samples was examined using a gaseous secondary electron detector (working pressure of 0.8 torr, acceleration voltage-30.00 KV) XL 30, (Philips, Netherlands)

Drug content and Encapsulation efficacy [7]:

The drug entrapment efficiency was calculated using the total drug content of liposome dispersion and unentrapped drug content of the dispersion. The total dug content of the dispersion is determined by adding sample Triton X-100 (0.001%) solution to disrupt the liposomal bilayer. The encapsulated drug gets liberated into the solution and the amount of the drug present in the whole solution was determined by UV-Visible spectrophotometer . Un encapsulated drug was determined by dispersing a weighed quantity (5ml) of liposome in phosphate buffer pH 7.4 Then the dispersion was centrifuged at 18000

rpm for 40 min at 50˚C . Later a clear fraction and cloudy fraction is clearly observed in the centrifugal tube , 1ml of clear fraction is used for the calculation of free drug in the formulation.

%Encapsulation Efficency= {1− (Unencapsulated Drug/total Drug)} ×100

FTIR –Spectroscopy:

Fourier transform infrared spectroscopy (FT-IR) is a simple technique for the detection of changes within excipient – drug mixture. Disappearance of an absorption peak or reduction of the peak intensity combined with the appearance of new peaks give a clear evidence for interactions between drug and excipient. FTIR spectra of drug and excipients were mixed in combinations and were obtained by the conventional KBr disc/pellet method. The sample was grounded gently with anhydrous KBr and compressed to form pellet. The scanning range was 400 and 4000 cm^{-1}.

Zeta potential

Zeta potential of the best formulation EF4 liposomes was measured using electrophoretic light scattering by a Malvern Zetasizer Nano ZS. The measurement was performed at 25 ℃ after appropriate dilution with distilled water. All of the measurements were repeated at least three times

In-vitro diffusion studies:

In vitro release studies on liposomes were performed by using Franz diffusion cell. The capacity of the receptor compartment was 15 ml. The area of the donor compartment exposed onto receptor compartment was 1.41cm^2. Cellophane membrane was soaked in phosphate buffer pH 7.4 for 1 hr before carrying the experiment. The dialysis cellophane membrane had been mounted between the donor and receptor compartment. A weighed quantity of liposomes placed on one side of the dialysis membrane. The receptor medium was phosphate buffer pH 7.4. The receptor compartment was enclosed by a vessel to take care of the temperature at 37˚C. Heat was provided by using a hot plate with a magnetic stirrer. The receptor fluid was stirred by a magnetic bead fitted to a magnetic stirrer. At every sampling interval, 3ml samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on every occasion and checked for absorbance in UV-Spectrophotometer.

Results and discussions:

Calibration curve of ketoconazole:

The standard curve of ketoconazole was obtained by taking the absorbance at wave length 270nm. The values are shown in the (Table 2). The standard curve of ketoconazole is plotted by taking concentration on X- axis & absorbance on Y-axis and the curve was shown in (Figure 1).

Table 2. The standard curve of ketoconazole is plotted by taking concentration

S.No	Concentration (µg/ml)	Absorbance
1.	0	0
2.	5	0.050
3.	10	0.100
4.	15	0.150
5.	20	0.196
6.	25	0.240

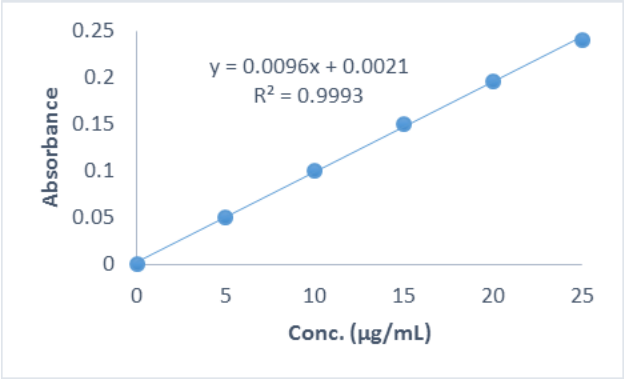

Figure 1. Calibration curve of ketoconazole

Optical microscopy:

The liposomes were found to be spherical and the pictures are shown in figures on 40 X magnification. The vesicles of Liposome under microscopy are shown below

Figure 2. Optical microscopy F1 Liposome.

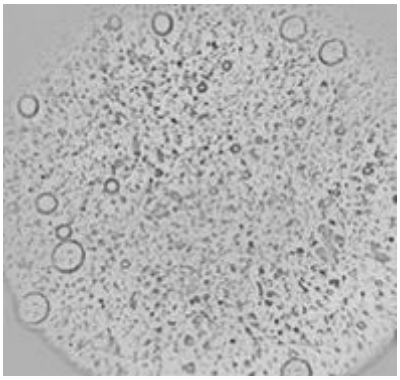

Figure 3. Optical microscopy F2 Liposome

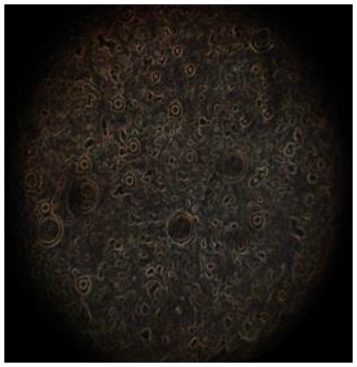

Figure 4. Optical microscopy F3 Liposome

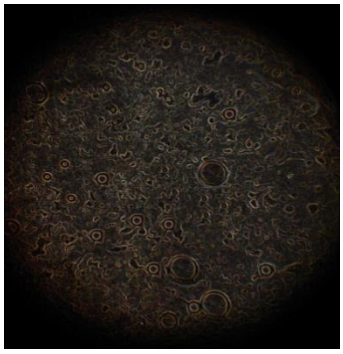

Figure 5. Optical microscopy E-F4 Liposome

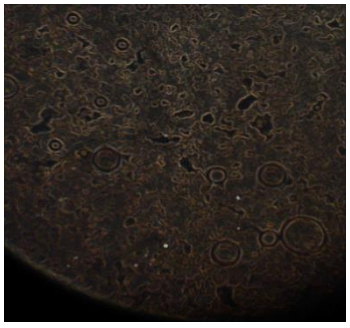

Figure 6: Optical microscopy E-F5 Liposome

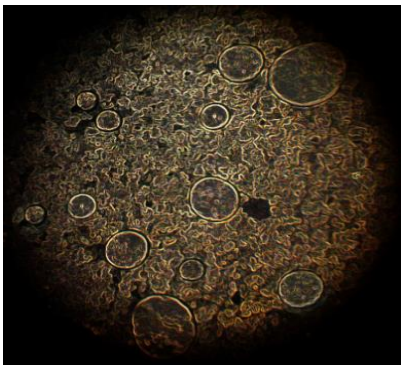

Figure 7. Optical microscopy E-F6 Liposome

Scanning electron microscopy

The SEM images of liposomes were found to be spherical. The size ranges from lowest to highest (149µm to 306µm) as shown in (Figure 8) for E-F4 and 136µm to 265µm for liposomes E-F5 as shown in (Figure 9).

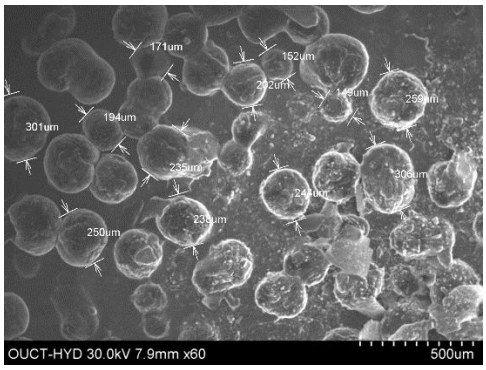

Figure 8. SEM image of E-F4 liposomes

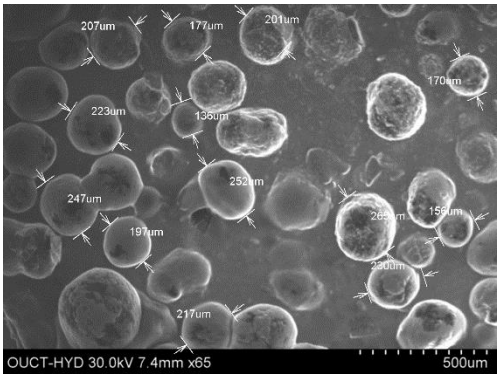

Figure 9. SEM image of E-F5 liposomes

Drug content and encapsulation efficiency of liposomes:

Among all the formulation E-F4 and E-F5 formulation shows the highest encapsulation efficacy and F3 and E-F6 shows the least encapsulation efficacy. The % Encapsulation efficiency of the formulations are listed in the Table : 3 and the result was shown in the (Figure 10).

Table 3. % Drug entrapped in ketoconazole Liposomes

Formulation	% Drug entrapped
F1	76.28
F2	82.7
F3	70
E-F4	92.36
E-F5	88.42
E-F6	70

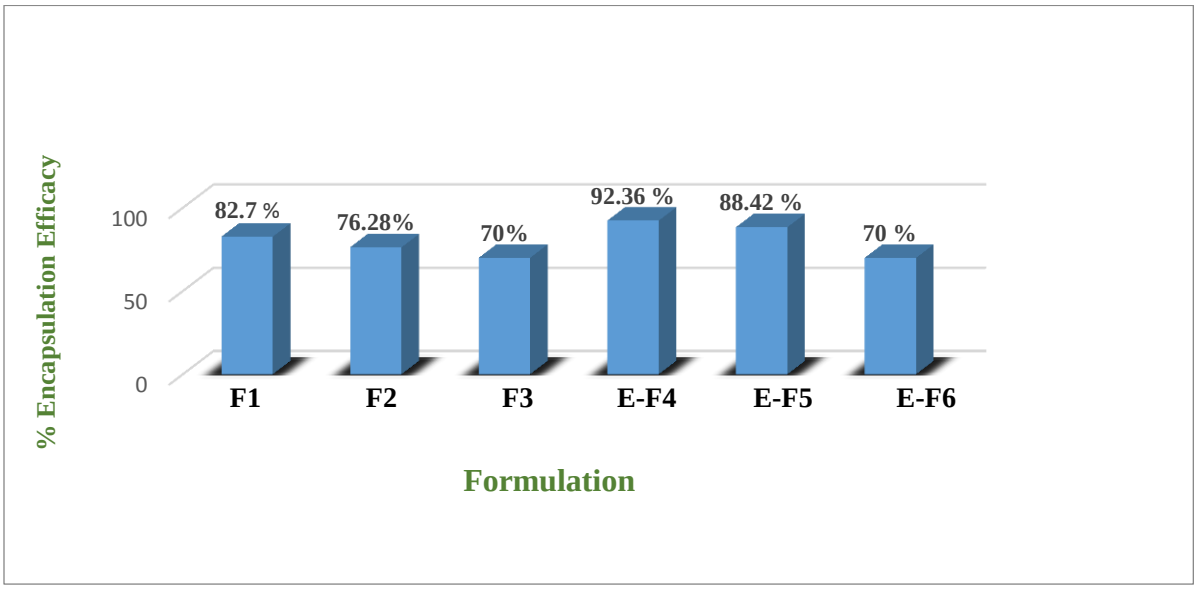

Figure 10: % Entrapment Efficacy

FTIR Studies

FTIR studies for ketoconazole pure drug, excipients and formulations E-F4 and E-F5 of liposomes are as shown in Table 4. From these it is concluded that there is no noticeable change in the peak of the spectrums of drug and excipients mixture. In recent observation there was no considerable variation in dosage stability and used excipients was found. Individual peaks of

ketoconazole were clearly established without any interaction of excipients used in the liposome formulation (E-F4) and (E-F5) as shown in the (Figure 13, 14).

Table 4: FTIR bands of ketoconazole liposomes

Functional group	**IR band of ketoconazole (cm^1)**	**IR band of ketoconazole Liposomes E-F4 & E-F5 (cm^{-1})**
C-C- stretch in aromatic	1470.97	1507.17,1466.78
Cl group present in aromatic ring	521.95, 483.84	554.14,563.05
C=O ketone stretching	1527.95	1635.90,1640.98
C-Cl- stretch	828.99	820.37,1740.97
Mono substituted benzene	670.08	693.19,722.36

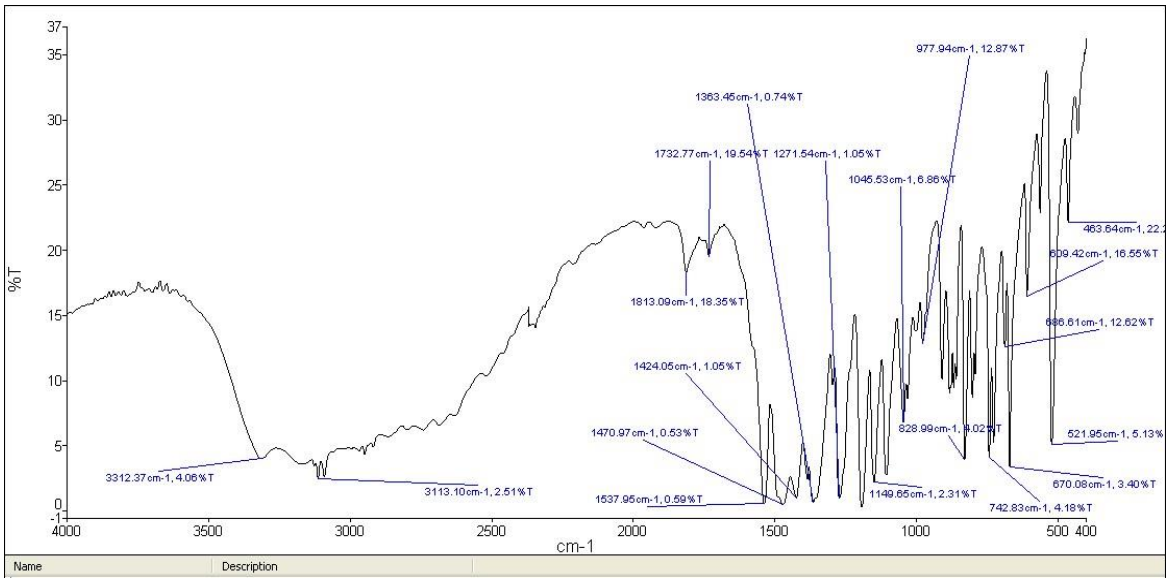

Figure 11. FTIR spectrum of ketoconazole pure drug.

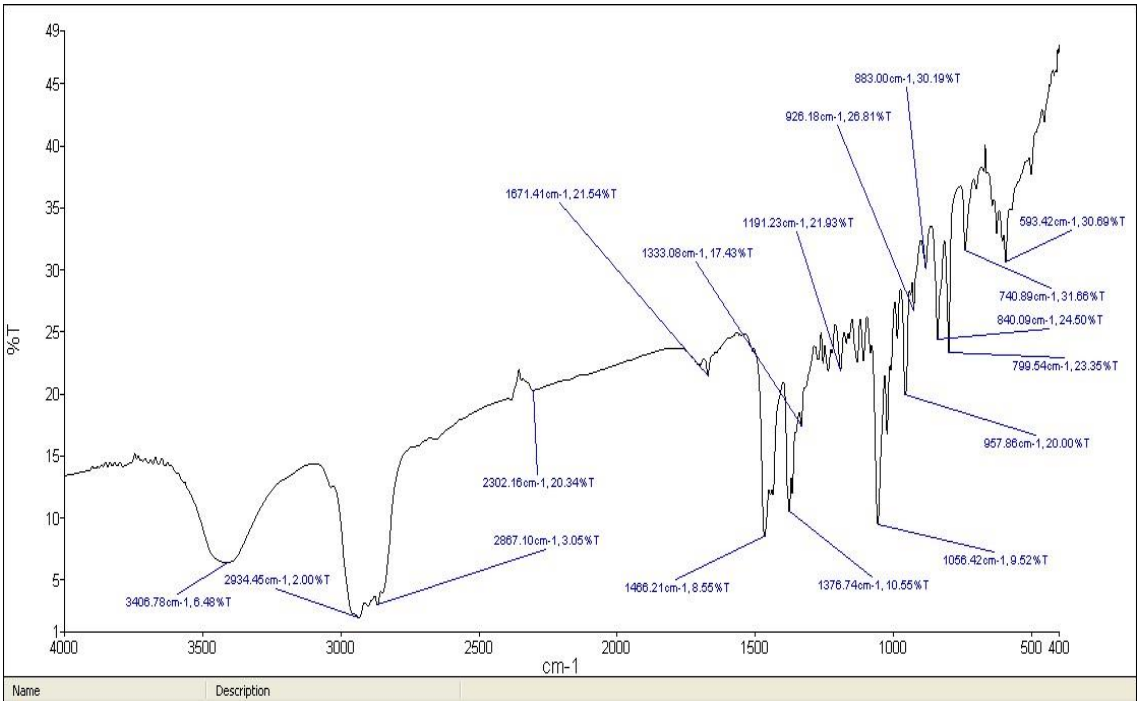

Figure 12: FTIR spectrum of cholesterol

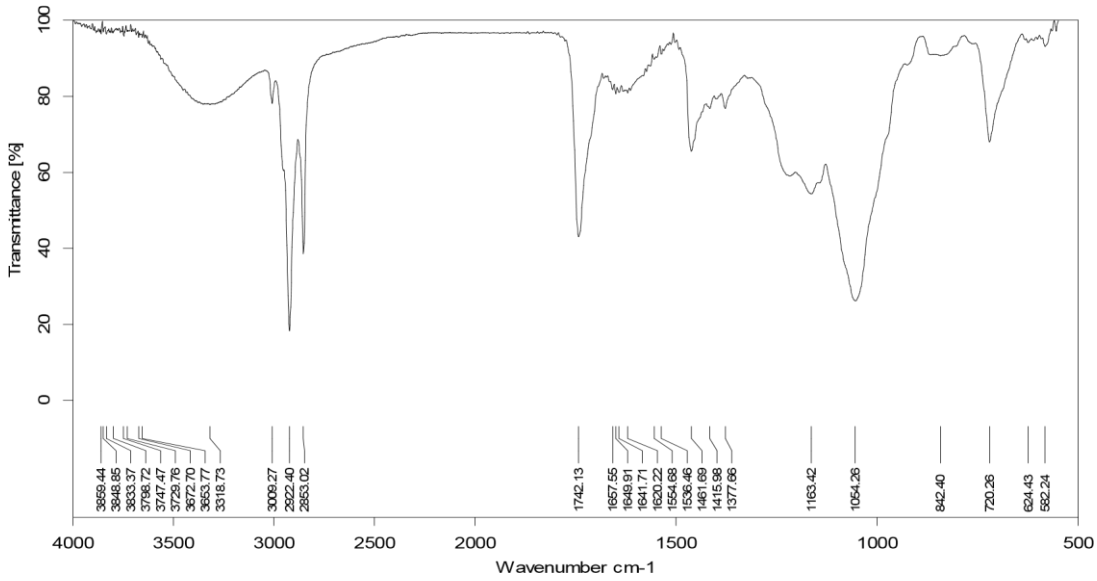

Figure 13a. FTIR spectrum of soya lecithin

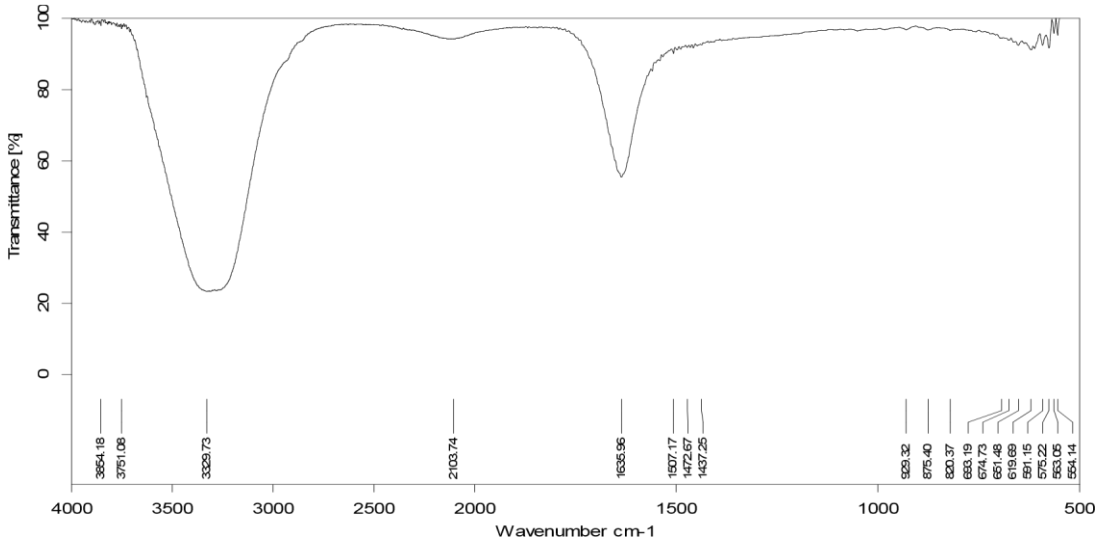

Figure 13b. FTIR of liposome formulation (E-F4)

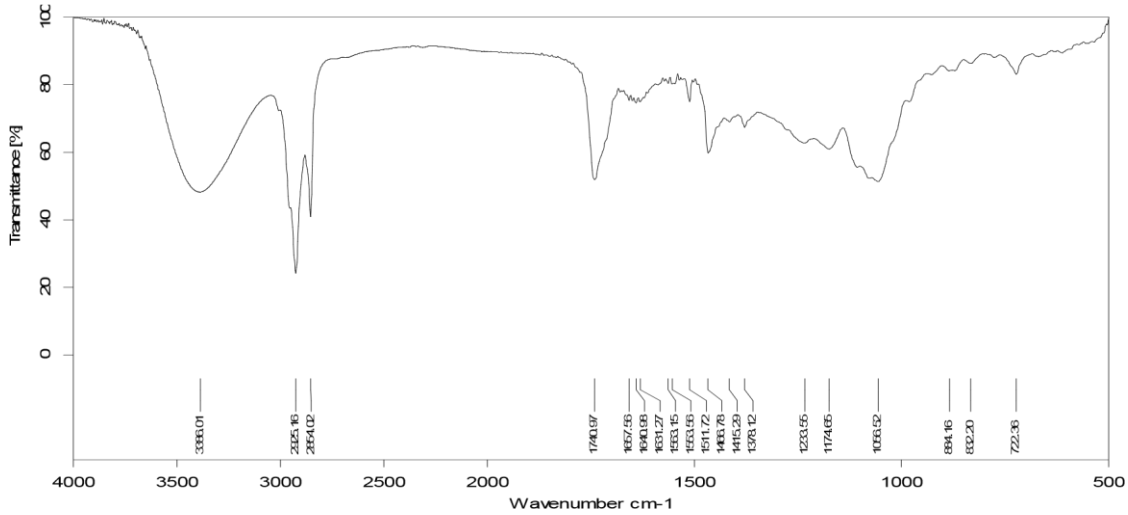

Figure 14: FTIR of Liposome formulation (E-F5)

Zeta Potential

The zeta potential indicates the degree of repulsion between, similarly charged particles in dispersion. For the particles that are small, a high zeta potential will offer stability and resist aggregation. When the potential is low leads to aggregation of particles and the dispersion will break and flocculate. The Zeta potential is the electrostatic charge of the particle surface which acts as a Repulsive energy barrier control in the stability of dispersion and opposing the proximity of particles and aggregation. The value of zetapotential 38.1v for the best formulation EF4 shows the stability of the formulation and shown in (Figure 15mV).

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Measurement Results

Measurement Results

Date	:	27 June 2016 12:44:04
Measurement Type	:	Zeta Potential
Sample Name	:	240
Temperature of the holder	:	25.1 deg. C
Viscosity of the dispersion medium	:	0.893 mPa.s
Conductivity	:	9.128 mS/cm
Electrode Voltage	:	2.2 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	139.5 mV	0.001082 cm2/Vs
2	-198.6 mV	-0.001541 cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	38.1 mV
Electrophoretic Mobility mean	:	0.000296 cm^2/Vs

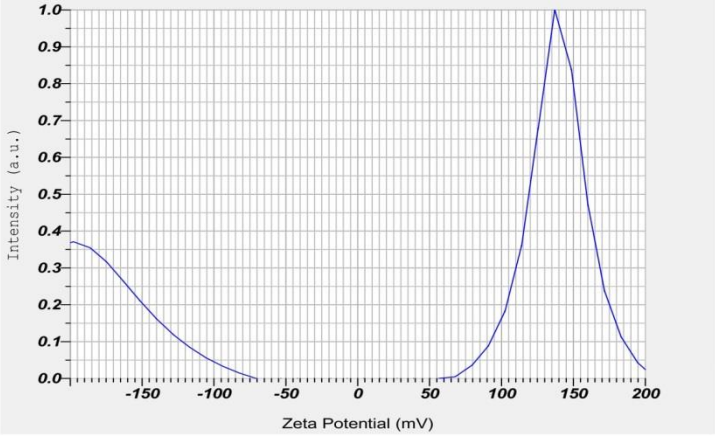

Figure 15. Zeta potential curve for the formulation EF4

***In vitro* release studies using dialysis membrane**

The release profile of liposomes are shown in (Figure 16) The release from all the formulation was slow and spread over more than 24 hrs and dependent on concentration and proportion of ingredients used in the formulations. Among all the preparations E-F4 shows highest drug release (81.84 %) and F2 shows lowest drug release (72.51 %).

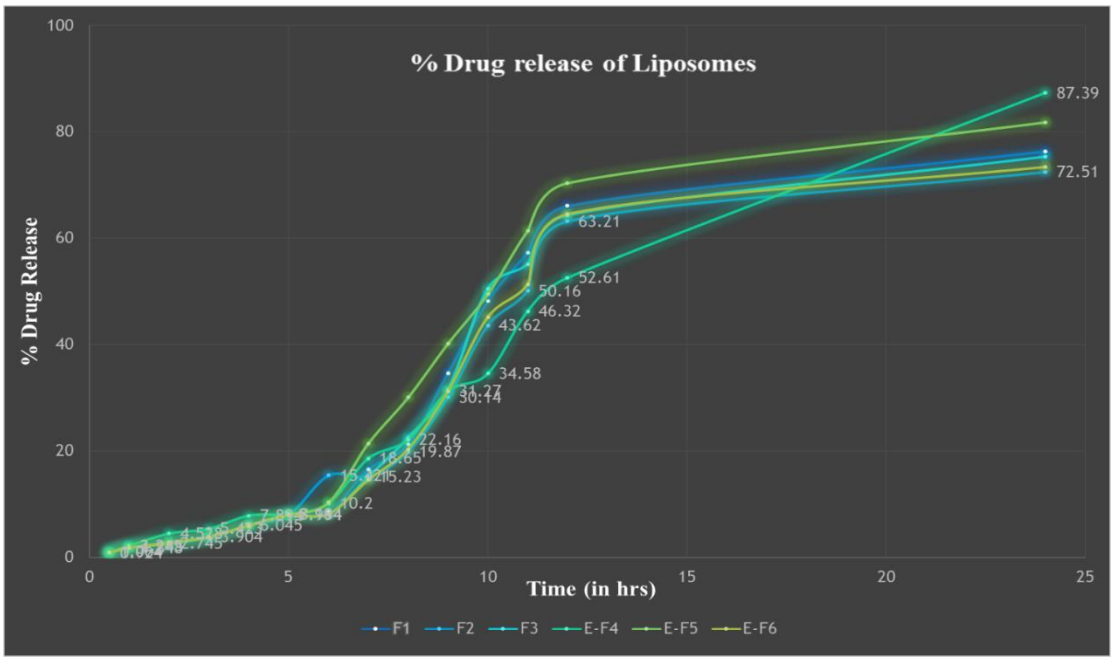

Figure16. % Drug Release of ketoconazole Liposomes

Summary and Conclusion

The objective of the present comparative study is to develop the vesicular system like liposomes for an anti-fungal drug ketoconazole for topical application and to enrich commercial Soya lecithin to increase phosphatidylcholine and to make a comparative study between the liposomes formulated with commercial soya Lecithin and Enriched Soya Lecithin. Ketoconazole liposomes were prepared by using soya lecithin and cholesterol. The preparation was prepared by film hydration technique. The prepared liposomes were evaluated for optical microscopy, size and size distribution, SEM analysis, Entrapment Efficacy, *In Vitro* Release Studies, and FTIR-Spectroscopy. Encapsulation efficiency of liposomes showed good amount of drug loading into the vesicles. Among all liposomal formulations E-F4 showed highest encapsulation efficiency 92.36 %. FTIR study proves that there is no interaction between pure drug and Excipients used in the formulation of Ketoconazole liposomes clearly established that without any interaction of excipients used in these formulation at 1507.17, 1466.78 (C-C- stretch in aromatic), 554.14, 563.05(Cl group present in aromatic ring), 1635.90, 1640.98 (C=O ketone stretching), 820.37, 1740.97 (C-Cl- stretch), 693.19, 722.36 (Mono substituted benzene). SEM images showed the size ranges from lowest to highest (149µm to 306µm) for E-F4 and 136µm to 265µm for liposomes E-F5. In-vitro diffusion studies for liposome's shows that highest percentage of drug release 87.39 % shown in 24 hrs for liposome formulation E-F4. Overall the study shows the liposomes prepared from enriched soya lecithin are better qualities in encapsulating the drug and drug release than commercial soya lecithin due to enrichment of phospholipids from commercial lecithin.

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