ISSN 0974-3618 (Print) **www.rjptonline.org**
0974-360X (Online)

RESEARCH ARTICLE

Formulation and *In vitro* Characterization of Ketoconazole Liposomal Gel for Transdermal Delivery

Jose Prakash. D*, Radhika Duggirala. L, Ariya. R, Thabitha Kumari
Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels University, Pallavaram, Chennai – 600117
*Corresponding Author E-mail:

ABSTRACT:
Liposomes are used because have many of the requirements for good drug delivery systems as they are relatively non-toxic and bio-degradable. And they help the ketoconazole to pass through the skin layers. Phosphatidyl choline and cholesterol were taken in different concentration. Liposomes are prepared by using thin film hydration technique. Gel containing carbopol 934 was prepared and characterization tests were performed for liposomal dispersion and liposomal gel. Liposomal gels were prepared with 1%, 1.5% and 2% carbopol gels which gave the clear idea for the efficient concentration of carbopol in topical gel.

KEYWORDS: Ketoconazole, liposomes, liposomal gel and transdermal delivery.

INTRODUCTION:

The threshold of a new era who improved delivery of a dosage forms to be an achievable goal that should result in improved survival and quality of life for the patients, without exposing that patient to an empirical trial or to possible morbidity or loss of time[1]. There are a number of carriers including proteins, glycoproteins, nucleic acids, starch particles, various synthetic polymers, and liposomes. The liposomes have been found to be useful carriers for both hydrophilic and hydrophobic drugs. Liposomal encapsulation of a drug can dramatically alter the pharmacokinetic properties of a drug, targeting the drug to particular organs and/or enhance the efficiency of the encapsulated drug[2].

Liposomes:

In 1960's liposomes has been used as a carrier to deliver a wide variety of compounds in its aqueous compartment[3, 4]. Phospholipids are dispersed in water, they spontaneously form closed structures with internal aqueous environment bounded by phospholipid bilayer membranes, and this vesicular statement is called liposome.

Liposomes are small vesicle of spherical shape that can be produced from cholesterols, non toxic surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane proteins. The classification of liposomes based on structure are unilamellar vesicles (Small and large) and Multilamellar vesicles (MLV).

Mechanism of Vesicle formation:

Liposomes are formed when thin lipid films or lipid cakes are hydrated and stacks of liquid crystalline bilayers become fluid and swell. The hydrated lipid sheets detach during agitation and self-close to form large, MLV which prevents the interaction of water with the hydrocarbon core of the bilayer at the edges. Once these particles have formed, reducing the size of the particle requires energy input in the form of sonic energy (sonication) or mechanical energy (extrusion) [5].

Application of Liposomes:

Liposomes interact with cells in many ways to cause liposomal components to be associated with target cells. The targeting of the liposome to the site of action takes place by the attachment of amino acid fragment, such as antibody or protein or appropriate fragments that target specific receptors cell[6, 7].

Received on 19.08.2016 Modified on 09.10.2016
Accepted on 04.11.2016
Research J. Pharm. and Tech. 2017; 10(1): 205-215.
DOI: 10.5958/0974-360X.2017.00044.0

Fungal Infections:
Fungi, the word for more than one fungus, can be found on different parts of the body[8]. Ketoconazole is used to treat Candida infection. Candida it is yeast, similar to a fungus. It most often affects the skin around the nails or the soft, moist areas around body openings. This is called yeast infection[9].

Ketoconazole is an Imidazole works by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effects on the host. Both fungi and humans are eukaryotes,so similar at the molecular level. This makes it more difficult to find or design drugs that target fungi without affecting human cells[10].

MATERIALS AND METHODS:

Materials:
Ketoconazole (Micro labs, Bangalore); Phosphatidyl Choline (Lipoid, Germany); Cholesterol (Hi Media, India); Carbopol 934 (Otto Chemika - Biochemika); Chloroform, Methanol, Sodium Chloride, Potassium Chloride, Disodium Hydrogen Phosphate dihydrate, Potassium Dihydrogen phosphate (Sisco Research Laboratories, India)

Liposome Preparation:
Take PC, Chol and ketoconazole in a 50 ml round bottom quick fit flask and dissolve in 10ml of chloroform: methanol (1:1). Flush the flask with nitrogen and maintain controlled vacuum. Rotate the flask to evaporate the solvent leaving a thin layer on the wall of round bottom flask. Keep the flask for 6 hrs to ensure complete removal of solvent system. Add 10ml of PBS. Seal the flask and hydrate the lipid film with manual shaking or using a manual shaker for 72 hrs. Centrifuge the liposomal suspension and discard the supernant. Redisperse in PBS and again centrifuge. Repeat the step 3-4 times to ensure the removal of unentrapped drug[11].
Formulation of Liposomes:

Table 1 - LD1 and LD2 - Liposomal Dispersion formulation 1 and 2 respectively

S.No.	Ingredients	Quantity LD1	Quantity LD2
1	Phosphatidyl Choline	1gm	1gm
2	Cholesterol	1gm	2gm
3	Ketoconazole	0.4gm	0.4gm
4	Chloroform	5ml	5ml
5	Methanol	5ml	5ml
6	Phosphate Buffer Saline	10ml	10ml

Characterization of Liposomes:

1. **Drug Entrapment Efficiency:**

Unentrapped drug from the prepared liposomes was separated by mini column configuration technique. Prepared liposomal suspension (0.2 ml) was placed in a Sephadex G-50 column and centrifuged at 2500 rpm for 3 min. Percent drug Entrapment (PDE) for the prepared liposomes were calculated by:

$$PDE = \frac{\text{Entrapped Drug (mg)} \times 100}{\text{Total Drug Added (mg)}}$$

2. **Scanning Electron Microscopy (SEM):**

The sample for the SEM analysis was to study the surface morphology and were prepared by applying a monolayer of the liposomes on to one side of the electron microscope brass stub and the stubs were then coated with gold in an ion sputter (JSM- T330A, JEOL, Japan). The pictures were taken with random scanning of the stub.

3. **Differential Scanning Colorimetry: (DSC)**

DSC is a thermo analytical technique to study the phase transitions of the drug and formulations. Sample of optimized formulation F1 is placed in aluminum pans and heated from 50^0 C to 350^0 C at a heating rate 10^0 C/min under inert atmosphere flushed with nitrogen at the rate of 20 ml/minute.

4. **Fourier Transform Infrared Spectroscopy: (FTIR)**

FTIR spectrometry is used to identify any interactions occuring after the preparation of formulation. The FTIR studies were carried out for Ketoconazole drug, carrier cholesterol and polymer carbopol alone and combinations of drug and carriers for the compatibility studies using Bomem FTIR MB II.

Incorporation of Liposomes into Gel:
Gel was prepared using carbopol 940 NF (1, 1.5 and 2%). The appropriate quantity of carbopol 940 powder was dispersed into distilled water under constant stirring with a glass rod, taking care to avoid the formation of indispersible lumps and allowed to hydrate for 24 h at room temperature for swelling. Topical liposome gel formulations were prepared by incorporation of liposome's containing Ketoconazole (separated from the unentrapped drug) were mixed into the carbopol gel with a mechanical stirrer (25 rpm, 2 m). The dispersion was neutralized using triethanolamine (0.5% w/w) [12].

Table – 2 Formulation of Liposomal Gel

S.No.	Ingredients	Quantity F1	F2	F3	F4
1	Phosphatidyl Choline	1gm	1gm	1gm	1gm
2	Cholesterol	1gm	2gm	1gm	1gm
3	Ketoconazole	0.4gm	0.4gm	0.4gm	0.4gm
4	Chloroform	5ml	5ml	5ml	5ml
5	Methanol	5ml	5ml	5ml	5ml
6	Phosphate Buffer Saline	10ml	10ml	10ml	10ml
7	Carbopol gel	1%	1%	1.5%	2%

Characterization of Liposomal Gel:

1. In vitro release studies:

The *in-vitro* drug release studies were conducted in pH 5.5 buffer for 8 hrs using Franz Diffusion Cell apparatus under sink conditions. Accurately weighed sample of the liposomal gel was taken in the donor compartment and 5.5 buffer was taken in receptor compartment with a magnetic bead in it. The whole setup was kept on a magnetic stirrer at optimum speed. At predetermined time intervals, aliquots were withdrawn, filtered and analyzed spectrophotometrically at 222 nm. The volume of dissolution medium was replaced immediately with an equal amount of phosphate buffer.

Table – 3 Drug transport mechanisms and diffusional exponents

Diffusional Exponent, *n*	Type of Transport	Time Dependence
0.5	Fickian diffusion	$t^{1/2}$
$0.5 < n < 1$	Anomalous transport	t^{n-1}
1	Case II transport	time independent
$n > 1$	Super case II transport	t^{n-1}

2. Drug Content:

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 222 nm using UV Visible spectrophotometer. Drug content was determined from calibration curve.

3. Viscosity:

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

4. pH:

The pH values of 1% aqueous solutions of the prepared gels were measured by a pH meter.

Release Kinetics:

The release data were analyzed on the basis of zero order, first order, Higuchi, Korsmeyers-Peppas R^2 values. Based on the diffusional exponent n value, the type of transport can be decided. The kinetics of ketoconazole liposomal gels was determined by finding the best fit of the release data to Zero Order Plot, First Order Plot, Higuchi, Korsmeyers-Peppas plots.

Plot of log Q_t/Q_∞ versus log time is taken and the slope of the plot gives *n* value. Korsmeyers-Peppas used this *n* value in order to characterize different release mechanisms. If the *n* value is 0.5 or less, the release mechanism follows Fickian diffusion, and higher values $0.5 < n < 1$ for mass transfer follow a non-Fickian model (anomalous transport). The drug release follows zero-order drug release and case-II transport if the *n* value is 1. For the values of *n* higher than 1, the mechanism of drug release is regarded as super case-II transport.

Comparision of Ketoconazole Liposomal Gel with Marketed Ketoconazole Cream:

As ketoconazole gel was not available in the market, marketed ketoconazole cream was taken(Nizoral 2%) and *in vitro* studies were conducted for the cream. These were compared with the optimized formulation and represented graphically

RESULTS AND DISCUSSION:

Ketoconazole liposomes are prepared by using the thin film hydration technique by using rotary flash evaporator. This is the most common technique used for the preparation of MLVs. Various characterization parameters are evaluated for ketoconazole liposomes and ketoconazole liposomal gel.

FT-IR Compatibility Studies:

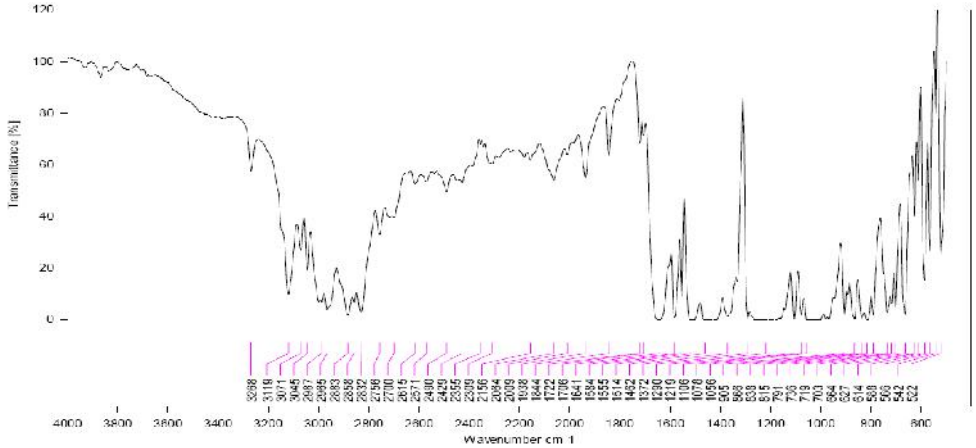

Figure 1 – FTIR graph of ketoconazole

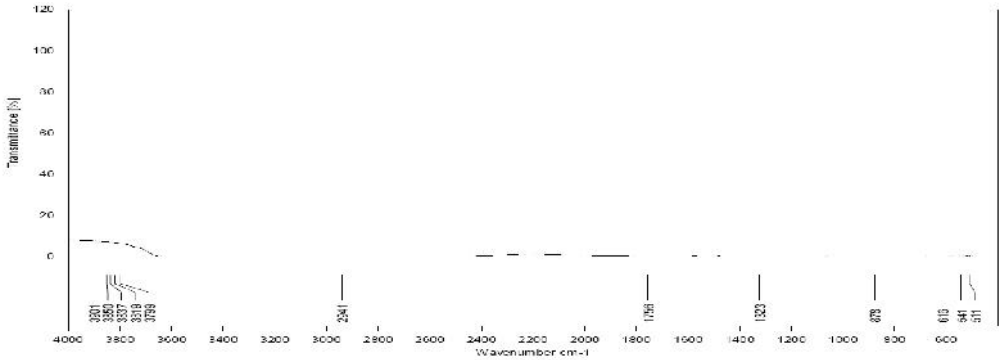

Figure 2 – FTIR graph of Carbopol 934

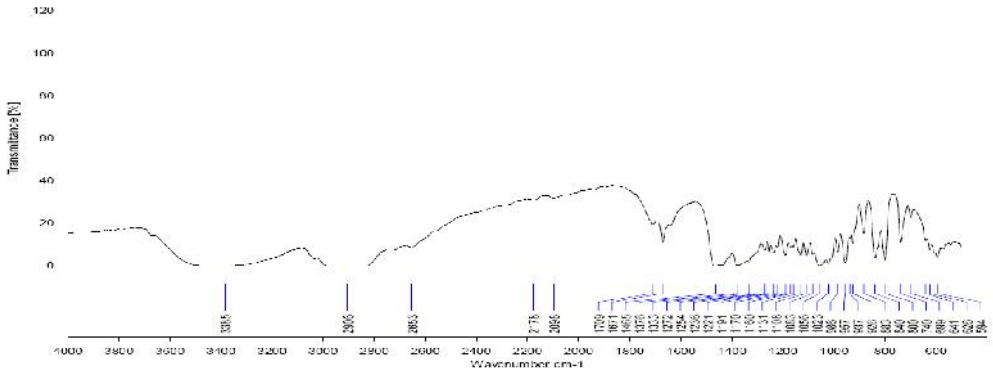

Figure 3– FTIR graph of Cholesterol

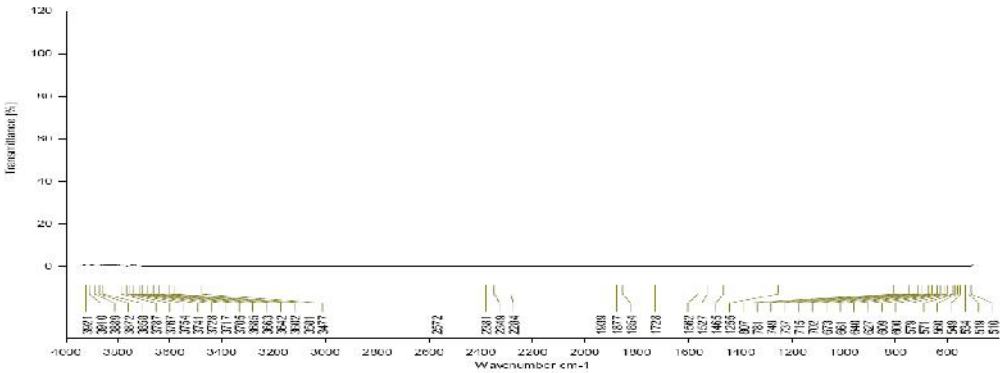

Figure 4 – FTIR graph of ketoconazole – Carbopol 934

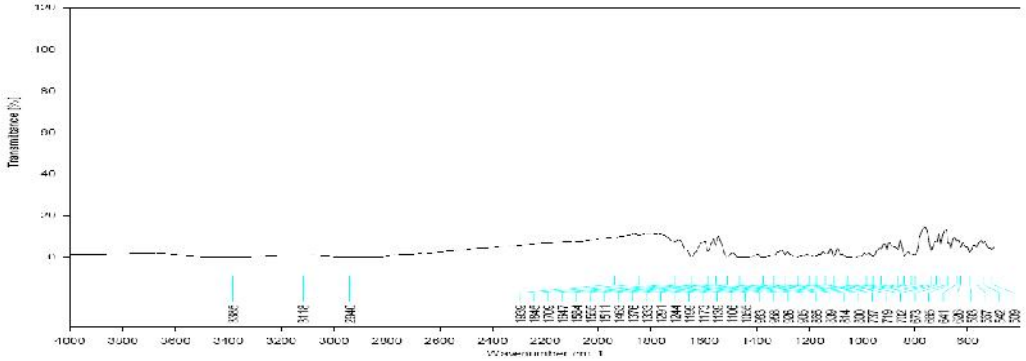

Figure 5 – FTIR graph of ketoconazole - Cholesterol

The wave numbers of individual FTIR spectrums of drug, polymer and carrier were similar to that of the wave number of FTIR spectrum of drug – polymer, drug – carrier combinations which indicates that there was no interactions between the drug, polymer and carrier used in the formulation.

Characterization of Liposomes:

1. Drug Entrapment Efficiency:

From the following results we can observe that the % drug entrapment efficiency is more for Liposomal Dispersion 1 (LD1) i.e., with phosphatidyl choline: cholesterol in 1: 1 ratio, rather than Liposomal Dispersion 2 (LD2) i.e., with phosphatidyl choline: cholesterol in 1: 2 ratio.

Table 4 - Drug Entrapment Efficiency of Liposomal Dispersion

S.No.	Formulation	% Entrapment Efficiency
1	LD1	96.35±0.05
2	LD2	91.5±0.13

Mean ± Standard Deviation (n = 3)

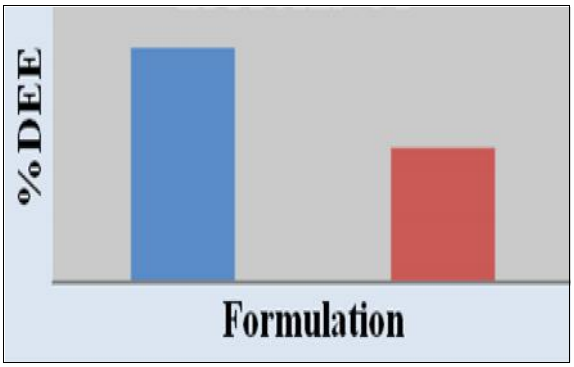

Figure 8 - Bar Graph for %Drug Entrapment Efficiency

1. Scanning Electron Microscopy (SEM):

SEM Analysis was performed for the optimized Liposomal Dispersion LD1 and it was found to be that the particle sizes of the multilamellar vesicles are optimum.

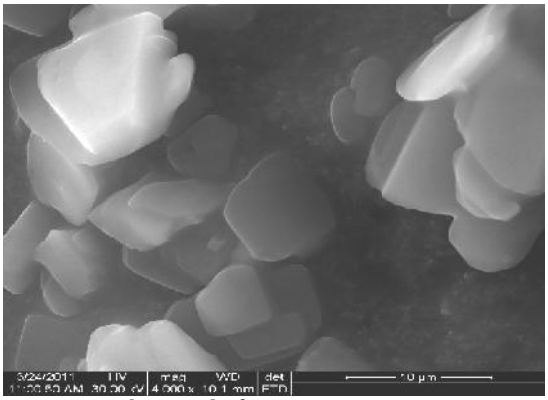

Figure 9 - SEM photograph of LD1

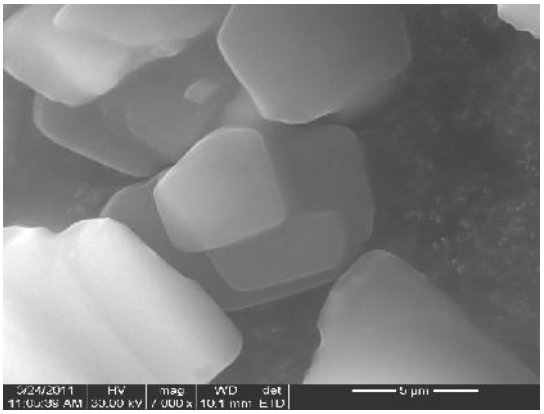

Figure 10 - SEM photograph of LD1

2. Differential Scanning Colorimetry:

DSC study was carried out for the optimized liposomal dispersion LD1 which shows the peak is not in between the melting point of ketoconazole which proves that the high entrapment efficiency of ketoconazole in the lipid bilayer.

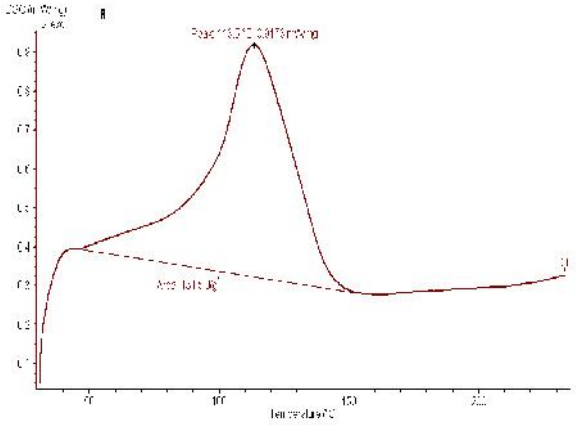

Figure 11 - DSC of Liposomal Dispersion

1. Fourier Transform Infrared Spectroscopy: (FTIR)

The FTIR studies are performed to the optimized Liposomal Dispersion (LD1). The wave numbers of Individual FTIR spectrums of drug and carrier were similar to that of wave number of FTIR spectrum of optimized Liposomal Dispersion (LD1), which indicates that there were no interactions in the dispersion. This is because the phosphatidyl choline and cholesterol interacts to form a bilayer. And as the ketoconazole is lipophillic drug, it entraps in the lipid bilayer but not reacting with the phosphatidyl choline and cholesterol.

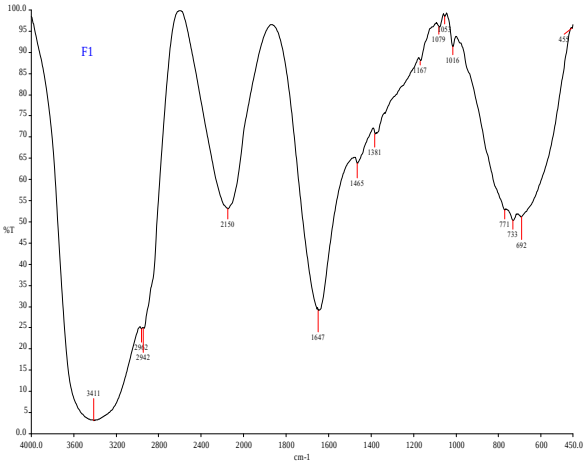

Figure 12 - FTIR graph for ketoconazole liposomal dispersio

Standard Curve for Ketoconazole:

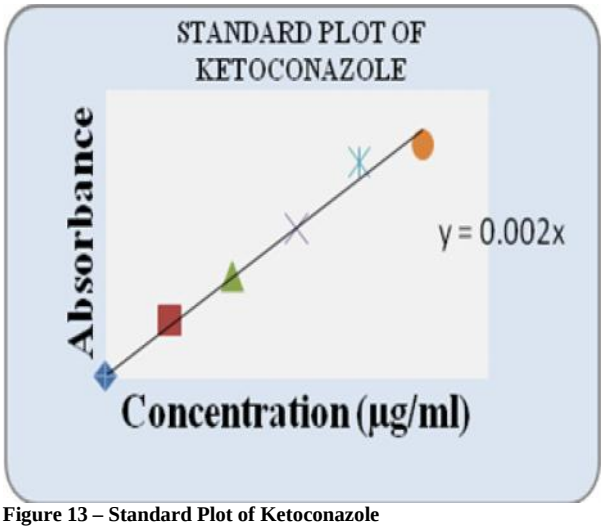

Figure 13 – Standard Plot of Ketoconazole

Characterization of Liposomal Gel:

1. In vitro release studies:

In vitro release studies for liposomal gels are performed using Franz Diffusion cell. Formulation F1 was found to be optimized formulation

%CDR – Cumulative Drug Release

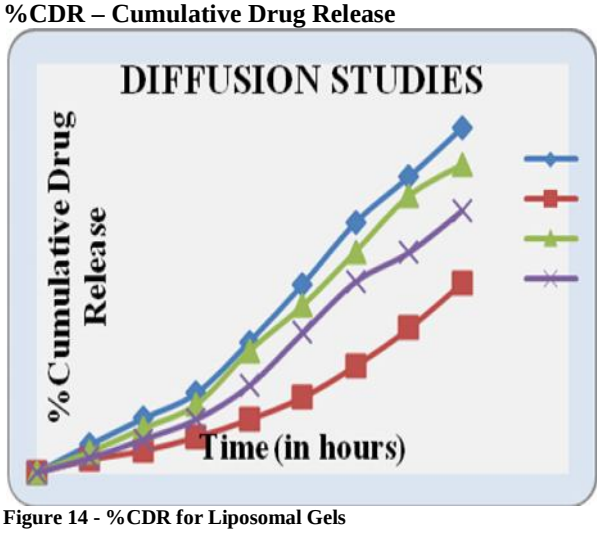

Figure 14 - %CDR for Liposomal Gels

Table 6 - %Drug Content of Liposomal Gels

F1	F2	F3	F4
96.35±0.01	91.55±0.54	92.25±0.23	93.506±0.296

Mean ± Standard Deviation (n = 3)

1. Drug Content (%):

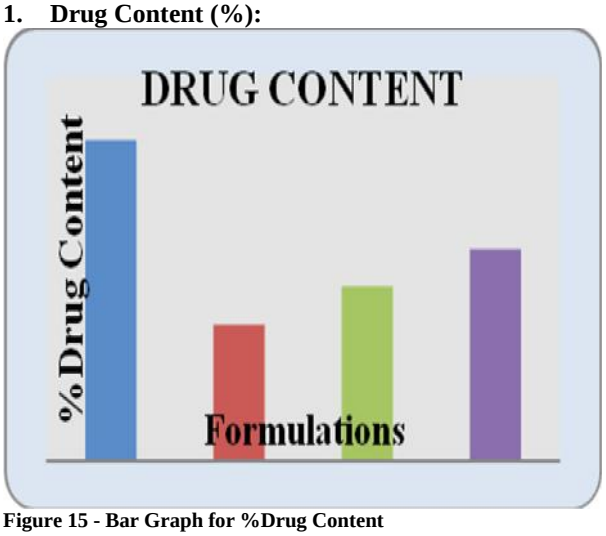

Figure 15 - Bar Graph for %Drug Content

Table 5 - %CDR for Liposomal Gels

S.No.	Time(hrs)	F1-%CDR	F2-%CDR	F3-%CDR	F4-%CDR
1	0	0	0	0	0
2	1	4.1±0.01	1.9±0.073	3.03±0.11	2.14±0.143
3	2	7.93±0.015	3.2±0.199	6.48±0.108	4.86±0.512
4	3	11.66±0.31	5.2±0.064	10.01±0.266	7.81±0.193
5	4	19.21±0.15	7.8±0.264	17.88±0.397	12.79±0.525
6	5	27.62±0.015	11.07±0.147	24.48±0.333	20.54±0.35
7	6	36.73±0.365	15.75±0.208	32.46±0.263	28.08±0.485
8	7	43.4±0.305	1.23±0.496	40.58±0.536	32.37±0.565
9	8	50.58±0.330	27.89±0.816	45.11±0.788	38.53±315

Mean ± Standard Deviation (n = 3)

2. Viscosity:

Table 7 - Viscosity of liposomal gels at different RPM

RPM	F1	F2	F3	F4
10	17580 cps	17590 cps	18120 cps	18340 cps
20	12090 cps	12110 cps	12540 cps	13310 cps
30	8440 cps	8390 cps	8930 cps	9140 cps
50	5940 cps	5910 cps	6740 cps	6860 cps
60	4330 cps	4330 cps	5910 cps	5250 cps
100	2790 cps	2720 cps	3120 cps	cps

3. PH:

Table 8 - pH of the liposomal gels (1% aqueous solution)

S.No.	Formulation	pH
1	F1	5.41 ± 0.53
2	F2	5.56 ± 0.47
3	F3	5.54 ± 0.90
4	F4	5.69 ± 0.77

RELEASE KINETICS:

Zero Order:

Figure 16 - Zero order plot for Liposomal Gels

First Order:

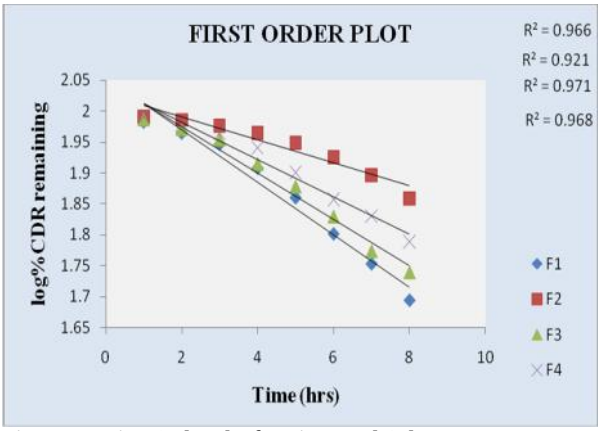

Figure 17 - First Order plot for Liposomal Gels

Higuchi Plot:

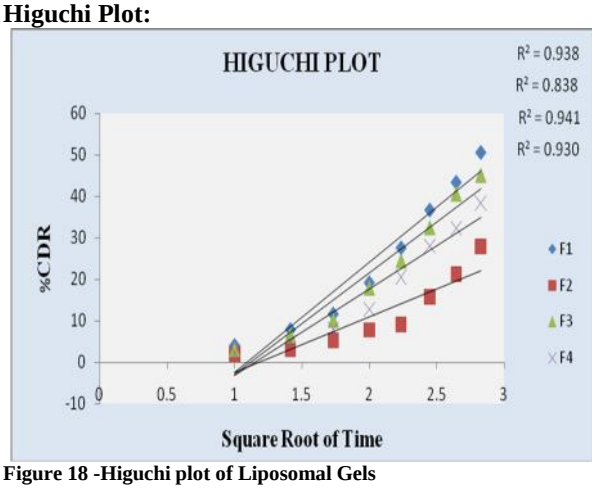

Figure 18 -Higuchi plot of Liposomal Gels

Korsmeyers-Peppas:

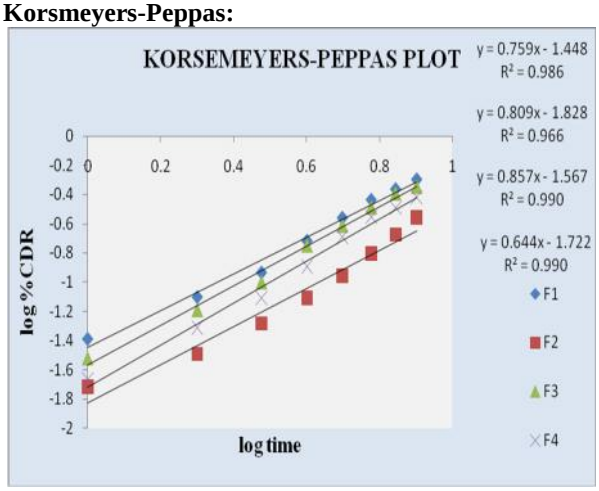

Figure 19 - Korsmeyers-Peppas Plot of Liposomal Gels

The release data were analyzed on the basis of Zero order, First order, Higuchi kinetics, and Korsmeyers-Peppas equation. The release rates *n* of each model was calculated by linear regression analysis using Microsoft Excel 2007 software. Coefficients of correlation (R^2) were used to evaluate the accuracy of the fit.

On the basis of calculations of the *n* values, the release mechanism was following the Non – Fickian Diffusion or Anomalous Transport which was best fitting to Zero order kinetics.

Table 9 – R^2 values of optimized formulation F1 for various plots

Optimized Formulation	Zero Order R^2	First Order R^2	Higuchi R^2	Korsmeyers – Peppas R^2	n	Mechanism
F1	0.920	0.966	0.938	0.986	0.759	Non – Fickian Diffusion Or Anomalous Transport

Comparision of Ketoconazole Liposomal Gel with Marketed Ketoconazole Cream:

In vitro studies were conducted for the marketed ketoconazole cream and compared with the optimized formulation of liposomal gel

Table 10 – Comparison of Diffusion profile of liposomal gel and marketed cream

S.No.	Time (hrs)	Marketed Ketoconazole Cream	Optimized Formulation (F1)
1	0	0	0
2	1	19.8 ± 0.11	4.1±0.01
3	2	30.6 ± 0.56	7.93±0.015
4	3	49.05 ± 0.66	11.66±0.31
5	4	62 ± 0.41	19.21±0.15
6	5	77.5 ± 0.19	27.62±0.015
7	6	93.45 ± 0.46	36.73±0.365
8	7		43.4±0.305
9	8		50.58±0.330

Mean ± Standard Deviation

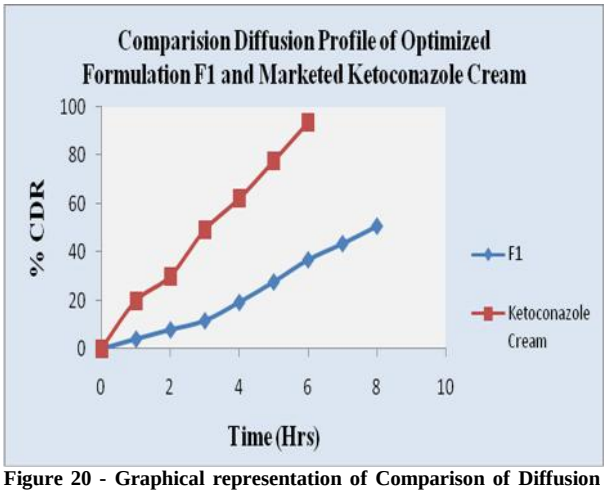

Figure 20 - Graphical representation of Comparison of Diffusion profile of liposomal gel and marketed cream

SUMMARY AND CONCLUSION:

- Conventional oral drug administration does not usually provide rate-controlled release. In many cases, conventional drug delivery provides sharp increase in drug concentration often achieving toxic level and following a relatively short period at the therapeutic level of the drug concentration eventually drops off until re-administration. The liposomal transdermal drug delivery is useful to give a prolonged controlled release of the drug.
- In present study Phosphatidyl Choline: Cholesterol is taken in 1:1 and 1:2 ratios. The drug entrapment efficiency was effective for 1:1. These 1:1 dispersions are incorporated into 1, 1.5 and 2% carbopol gels in which 1% carbopol gel was found to be with effective release.
- The liposomal dispersion LD1 and LD2 are evaluated, %DEE was found to be more for LD1 (96.35%). In formulations F1, F2, F3 and F4, F1 was found to optimized formulation with effective drug release (50.58%), Drug content was 96.35%. The SEM photograph showed the surface morphology of liposomes and particle size was found to be optimum so that it can be pass through the skin.
- The optimized formulation %CDR (50.58% after 8hrs) is compared with the marketed ketoconazole cream %CDR (93.45% after 6 hrs) which confirms that liposomal gel gives the prolonged controlled release than the ordinary conventional formulations.
- So from the obtain results, the F1 formulation was found to be optimized formulation with prolonged release and good entrapment efficiency.

ACKNOWLEDGEMENT:

I would like to express my sincere gratitude to Vels University (VISTAS) and its management for providing research facilities and encouragement for the continuous support of my research,

REFERENCES:

1. Yie W. Chein, Novel Drug Delivery Systems, Second Edition, Revised and Expanded, Marcel Dekker, INC. New York, Basel, Hong Kong, 1-2, 301-380.
2. Joseph R. Robinson, Vincent H. L. Lee, Controlled Drug Delivery fundamental and applications, Second Edition, Revised and Expanded, Marcel Dekker, INC. New York, 3-61.
3. Abdus Samad, Y. Sultana and M. Aqil, Liposomal Drug Delivery Systems: An update review, *Current Drug Delivery*, 2007; 4: 297-305.
4. Vladimir P. Torchilin and Volkmar Weissing, Liposomes practical Approach, Second Edition, Oxford University, 3-30.
5. Mechanism of vesicles formation and Method of Preparation of Liposomes, http://www.Avanthilipids.com
6. Applications of Liposomes to different routes of administration, http://www.google.com
7. Vyas S. P., Khar R. K., Targeted and Controlled Drug Delivery-Novel Carrier Systems, First Edition, CBS Publications and Distributors, 173-243.
8. Satoskar R. S., Bhandarkar S. D., Ainapure S. S. Pharmacology and Pharmacotherapeutics, Revised 17th Edition, Popular Prakashan, 2001, 675.
9. David J. Hauss, Oral Lipid-base Formulations, *Advanced Drug Delivery Reviews*, 2007; 59: 667-676.
10. Constantinides P. P, Lipid microemulsions for improving drug dissolution and oral absorption: Physical and Biopharmaceutical Aspects, *Pharm. Res.* 1995; 12: 1561-1572.
11. S. P. Vyas, Theory and practice in Novel Drug Delivery Systems, Page No. 174.
12. B. V. Mitkari , S. A. Korde , K. R. Mahadik and C. R. Kokare, Formulation and Evaluation of Topical Liposomal Gel for Fluconazole, *Indian J.Pharm. Educ. Res.*, 2010; 44(4): 324-333.