

# Design, development and evaluation of ethosomal gel of fluconazole for topical fungal infection

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**ABSTRACT:** In the present investigation efficiency of ethosomes as novel lipid carriers for topical delivery of fluconazole has been evaluated. Ethosomes were optimized by varying concentration of phospholipid and ethanol. Ethosomal formulation (F8) with soya phosphatidylcholine (3%) and ethanol 20% was optimized. On characterizations spherical, unilamellar vesicles with smooth surface were observed under transmission electron microscopy (TEM) in the range of 5 to 200 nm. Zeta potential of F8 formulation was found to be - 4.16 mv. Drug entrapment efficiency of F8 formulation was found to be 90 %. The optimized formulation exhibited pH (8) and viscosity (73,200 cps). Physical evaluation of ethosomal gel was done. *In vitro* release of F8 formulation was carried out which showed  $92 \pm 0.79$  % release over a period of 8 hours. Skin irritation study revealed no irritation from ethosomal gel. From the data obtained after plotting various models it was observed that the Higuchi model was found to be best suited with  $R^2$  value of 0.9678. Stability studies performed at  $40^\circ\text{C} \pm 1^\circ\text{C}$  and  $25^\circ\text{C} \pm 1^\circ\text{C}$  for 3 months showed good storage stability. No phase separation was observed in the optimized ethosomal gel. Results suggested that ethosomes as efficient carriers for fluconazole topical delivery.

**Keywords:** Vesicle, fluconazole, *in vitro* release, transmission electron microscopy, topical delivery

## Introduction

Over the past years, topical delivery of drug has caused more and more attention; this has the advantage that high concentration of drugs can be localized at the site of action, reducing the systemic side effects compared to parenteral or oral drug administration. Topical delivery can be defined as the application of a drug containing formulation to the skin to treat cutaneous disorders (e.g. psoriasis, acne) with the intent of containing the pharmacological or another effect of the drug to the surface of the skin or within the skin. Intensive research over the past two decades led to the development of novel carriers, the ethanolic liposomes that have been termed ethosomes (Touitou *et al.*, 2000).

Ethosomes are novel lipid carriers composed of ethanol, phospholipids and water.

These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are reported to improve the skin delivery of various drugs. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization. Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic. The size range of ethosomes may vary from tens of nanometers to microns.

The present investigation was to design the ethosomal gel containing fluconazole using different concentration of ethanol and phospholipid. Fluconazole is a polar *bis*-triazole antifungal drug. Studies have shown that fluconazole exhibits specificity as an inhibitor of the fungal as opposed to mammalian cytochrome P-450 mediated reactions, including those involved in steroid biosynthesis and drug metabolism. Fluconazole is used in treatment of candidiasis seen in the oral cavity, pharyngeal, vaginal and urinary tract infection.

It is a BCS class III drug (high solubility and low permeability). On oral administration, its bioavailability is low due to poor aqueous solubility. Fluconazole was selected for formulation of topical ethosomal gel because patients with diseases candidiasis and urinary tract infection etc., the dose is given at a higher level due to its low permeability. The ethosomal approach was selected to enhance the permeability of fluconazole that increase bioavailability, reduce the side effects, reduce large doses and increase the therapeutic efficacy.

In the present study, ethosomes bearing fluconazole was prepared by cold method and

optimized. They were evaluated for permeation enhancement over marketed formulation and the optimized formulation (F8) for vesicle skin interaction studies and stability studies.

### Material and method

Fluconazole (FLZ) was obtained as a gift sample from Belco Pvt. Ltd., India. Soya lecithin was obtained as a gift sample from Hi Media laboratories Pvt. Ltd. Mumbai, India. Ethanol was purchased from Loba Chemie Pvt. Ltd., Mumbai, India.

### Preformulation studies of drug sample

Preformulation study is the first step in the rational development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms. Following preformulation studies were performed.

### Organoleptic properties

The organoleptic studies like general appearance like nature, color, odor, etc. were performed by visual observations and compared with standard of drug given in pharmacopoeia for identification of drug.

**Color:** Small quantity of drug was taken on butter paper and viewed in well illuminated place.

**Odor:** Very less quantity of drug was smelled to get the odor.

### Solubility studies

Semi quantitative determination of the solubility was made by adding solvent to glass tube containing accurately weighed amount of solute. The system is vigorously shaken and examined visually for any undissolved solute particles. The solubility is expressed in terms of ratio of solute and solvent. The solubility study of Fluconazole was performed in methanol, ethanol, acetone, hexane, ether, chloroform, propylene glycol, distilled water, 0.1 N HCL, phosphate buffer

solution pH 5.5, 6.8, 7.4, separately by keeping the drug containing test tube on vortex mixture.

### Determination of melting point

For determination of melting point USP method was followed. Small quantity of drug was placed into a sealed capillary tube. The tube was placed in the melting point apparatus. The temperature in the apparatus was gradually increased and the observation of temperature was noted at which drug started to melt and the temperature when the entire drug gets melted was noted.

### Determination of partition co-efficient<sup>(85)</sup>

The known quantity of fluconazole was added into 20 ml of octanol and it was mixed with 20 ml of phosphate buffer pH 7.4 in a separating funnel. Then two phases were allowed to equilibrate at 37 °C for 2 hours with intermittent shaking. The concentration of drug in the aqueous phase and organic phase was determined by UV spectroscopic method at  $\lambda_{max}$  260 nm after necessary dilution. The apparent partition coefficient was calculated as the ratio of drug concentration in each phase by the following equation -

$$K_p = \frac{C_{organic}}{C_{aqueous}}$$

**C<sub>organic</sub>** is concentration of drug in organic phase

**C<sub>aqueous</sub>** is concentration of drug in aqueous phase

### Determination of drug pH

The pH of Fluconazole was determined using digital pH meter for freshly prepared 1% solution of Fluconazole in methanol.

### Infrared spectroscopic analysis

The Fourier infra red spectrums of moisture free samples of fluconazole, soya lecithin, carbopol, methyl paraben, propyl paraben and mixture of fluconazole, carbopol, methyl paraben, propyl paraben were recorded on IR spectrophotometer. Infrared spectroscopy of different compounds was performed for identification of that particular compound. FTIR Spectroscopy was done using KBr pellets. Various peaks in FTIR spectrum were

interpreted for identification of different group in the structure of fluconazole. FTIR Spectroscopy can also be used to investigate and predict any physicochemical interactions between different components. The scanning range varies from 4000 – 400  $\text{cm}^{-1}$  and the resolution was 1  $\text{cm}^{-1}$ .

### **Analysis by UV-Visible spectrophotometry Preparation of standard curve in methanol**

#### **Standard stock solution of fluconazole**

Accurately weighed 100 mg of fluconazole and was dissolved in 100 ml of methanol, from this stock solution 10 ml was withdrawn and transferred into 100 ml volumetric flask. Volume was made with methanol in order to get standard stock solution containing 100  $\mu\text{g/ml}$ .

#### **Standard graph of fluconazole**

From this standard stock solution, a series of dilution (10, 20, 30, 40, 50  $\mu\text{g/ml}$ ) were prepared using methanol. The absorbance of these solutions was measured spectrophotometrically against blank of methanol at 260 nm for fluconazole.

#### **Determination of wavelength maxima of fluconazole**

The solution was scanned in the range of 200 to 400 nm to fix the maximum wavelength and UV spectrum was obtained.

#### **Method of preparation of ethosomes of fluconazole**

Ethosomal formulations were prepared by using the cold method. The ethanolic vascular system was composed of phospholipid (2.0% to 4%

W/V), ethanol (20% to 40% V/V), propylene glycol (20 % V/V), drug (fluconazole, 0.5% W/V) and distilled water to 100% (V/V). Phospholipid was dissolved along with the drug in ethanol. This mixture was heated to  $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and a fine stream of distilled water was added slowly, with constant mixing at 700 rpm with a mechanical stirrer in a closed container. Mixing was continued for an additional 5 minutes, while maintaining the system at  $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The preparation was left to cool at room temperature for 30 min and then it was sonicated at  $4^{\circ}\text{C}$  for five cycles of 3 minutes each with a minute rest between cycles using a probe sonicator.

Nine formulations were prepared using different concentration of phospholipid and ethanol among them optimized formulation was selected for characterization and evaluation studies.

Table: Compositions of different ethosomal formulation of fluconazole

Formulation code	Conc. of Phospholipid (W/V)	Conc. of Ethanol (V/V)	Conc. of Propylene glycol (V/V)	Conc. of drug (W/V)	Conc. of distilled water (V/V)
F 1	2 %	20 %	20 %	0.5 %	Upto 100%
F 2	3 %	30 %	20 %	0.5 %	Upto 100%
F 3	4 %	40 %	20 %	0.5 %	Upto 100%
F 4	2 %	30 %	20 %	0.5 %	Upto 100%
F 5	3 %	40 %	20 %	0.5 %	Upto 100%
F 6	4 %	20 %	20 %	0.5 %	Upto 100%
F 7	2 %	40 %	20 %	0.5 %	Upto 100%
F8	3 %	20 %	20 %	0.5 %	Upto 100%
F 9	4 %	30 %	20 %	0.5 %	Upto 100%

### Preparation of the carbopol gel<sup>(87)</sup>

Carbopol934 forms very good consistency transparent gel at low concentration. 1% carbopol gel base was prepared by dispersing 1 g carbopol934 in 90 ml hot distilled water in which 10 ml glycerol was previously added. Accurately weighed quantity of methyl paraben and propyl paraben was also added into it. The mixture was stirred until thickening occurred and then neutralized by the drop wise addition of 50% (w/w) triethanolamine to achieve a transparent gel.

Table: Composition of the gel base

Ingredients	Concentration
Carbopol 934	1%
Glycerol	5%
Methyl paraben	0.02%
Propyl paraben	0.01%
Distilled water	Upto 100%

### Incorporation of ethosomes in the gel base

The ethosomal formulation was slowly added in carbopol 934 gel base with gentle stirring. Finally, the ethosomal gel was mixed using a mechanical stirrer for 5 min.

### Evaluation of ethosomes

#### Drug entrapment efficiency

The total volume of the ethosomal suspension was measured. 5ml of this formulation was diluted with distilled water up to 8 ml and centrifuged at 15,000 rpm for 45 min at 4<sup>0</sup>C using a cooling centrifuge. After centrifugation, the supernatant and sediment were recovered, their volume was measured. Then sediment was lysed using n- propanol and filtered through a 0.45 µm nylon disk filter. The concentration of fluconazole in the supernatant and sediment was analyzed by UV-spectroscopic method at 260 nm. The percent drug entrapment was calculated using the following equation:

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of entrapped drug recovered}}{\text{Total amount of drug}} \times 100$$

### **Vesicular shape and surface morphology**

Ethosome vesicles were visualized using transmission electron microscopy (TEM Philips Technai electron microscope, Netherlands). A drop of ethosomal solution was dried on a microscopic carbon coated grid, to get adsorbed and the surplus was removed by filter paper. A drop of 1% aqueous solution of phosphotungstic acid (PTA) was then added and left in contact with the sample for 5 minutes. The excess solution was removed and the sample was dried at room condition before the vesicles were viewed under TEM operating at an acceleration voltage of 200 KV.

### **Vesicle size measurement**

The vesicles size was determined by dynamic light scattering method (DLS), using a computerized inspection system (Malvern Zetasizer Nano-ZS, Malvern, U.K.) with DTS<sup>®</sup> (Nano) software. For vesicles size measurement, vesicular suspension was diluted with distilled water and put into the cuvetts of zetasizer. Then the measurements were conducted at 25<sup>0</sup>C. The DLS measurements were performed over alternating increasing and decreasing temperature cycles at each temperature the sample was equilibrated for at least 3 minutes before performing the measurement. The average hydrodynamic diameter of the ethosomes under consideration corresponds to the Z-average value measured by DLS. Hence, the data was collected for vesicles size and size distribution.

### **Zeta potential measurement**

Zeta potential is the measure of the magnitude of the electrostatic or charge repulsion or attraction between particles and, known to affect stability. Its measurement brings detailed insight into the causes of dispersion, aggregation or flocculation, and can be applied to improve the formulation of ethosomes. Almost all particulate or macroscopic materials in contact with a liquid acquire an electronic charge on their surfaces. In general, particles could be dispersed stable when the absolute value of zeta potential is above 30 mV. Moreover, the zeta potential below 20mV is of limited stability and that below 5mV show rapid aggregation. However, several studies have

reported that the zeta potentials of ethosome formulations ranged between -10 to -20 mV. Zeta potential of ethosomal formulation was determined using Zeta Sizer (Nano-ZS, Malvern, U.K.) at 25<sup>0</sup>C.

### **Physical evaluation of ethosomal gel**

The ethosomal gel formulation of fluconazole was evaluated for organoleptic characteristics, occlusiveness and washability.

### **Measurement of pH of the ethosomal gel**

1 g fluconazole ethosomal gel was mixed in 100 ml distilled water with homogenizer. Then the electrode was immersed in the prepared gel solution and readings were recorded from digital pH meter in triplicate and average value was calculated.

### **Viscosity study**

Viscosity measurements were done on Brookfield viscometer by selecting suitable spindle number and rpm. 50 g of preparation was kept in 50 ml beaker which was set till spindle groove was dipped and rpm was set and dial reading was measured after three minutes. From the reading obtained, viscosity was calculated by using factor. The procedure was repeated three times and observations are recorded as mean.

### **Spreadability**

It is the term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula:

$$S = M \cdot L / T$$

Where M = wt. tied to upper slide

L = length of glass slides

T = time taken to separate the slides

0.1 g of ethosomal gel was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 minutes where no more spreading

was expected. Diameters of spreaded circles were measured in cm and were taken as comparative values for spreadability. The standardized weight tied on the upper slide was 125gm. The results obtained are average of three determinations.

### **Extrudability study**

The extrudability of ethosomal gel was determined by filling ethosomal gel in the collapsible tubes. The extrudability of the ethosomal gel was determined in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 second.

### **Percentage yield**

The empty container was weighed in which the ethosomal gel formulation was stored then again the container was weighed with ethosomal gel formulation. Then subtracted the empty container weighed with the container with gel formulation then it gives the practical yield. Then the percentage yield was calculated by the formula.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

### **Homogeneity and grittiness**

A small quantity of ethosomal gel was pressed between the thumb and the index finger. The consistency of the ethosomal gel was noticed (whether homogeneous or not), if there was any coarse particles appeared on fingers. Also, the homogeneity could be detected when a small quantity of the ethosomal gel was rubbed on the skin of the back of the hand. The grittiness of prepared ethosomal gel was also observed in the same manner.

### **Skin irritation study**

Skin irritation study was carried out on healthy rats (150-200 g) of either sex. The animals are maintained on standard animal feed and had free access to water. Before one day of starting the study hair was shaved from back of rats and area of 5 cm<sup>2</sup> was marked on both the sides, one side served as control while the other side was test.

Prepared ethosomal gel was applied for 7 days and the site was observed for any sensitivity and the reaction if any, was graded as 0, 1, 2, 3 for no reaction, slight patchy erythema, slight but confluent or moderate but patchy erythema and severe erythema with or without edema, respectively.

### ***In vitro* release studies Skin permeation studies**

Franz diffusion cell was used for permeation studies. Study was conducted using prepared rat skin. 28 ml of PBS 7.4 was taken in receptor compartment and was continuously stirred with a magnetic stirrer and equilibrated at 37<sup>o</sup>±1<sup>o</sup>C with a recirculating water bath. The prepared skin was mounted with stratum corneum facing upward into the donor compartment. 1 g of ethosomal gel formulation was taken in donor compartment and covered with parafilm to avoid any evaporation process. 5 ml sample was withdrawn through the sampling port at predetermined intervals over 8 hours and each sample was replaced with equal volume of fresh dissolution medium. Then the samples were analyzed for drug content by using phosphate buffer as blank with UV-Visible double beam spectrophotometer at 260 nm. Similar study was performed with marketed fluconazole gel.

### **Franz diffusion cell**

*In vitro* absorption studies are generally carried out in vertical Franz diffusion cell. According to Food and Drug Administration (FDA) regulations, it is an ideal tool for quality control of topical preparations. It has a receptor and a donor chamber, which is filled with phosphate buffer medium. The schematic diagram of a vertical FDC is shown in figure.

It consists of a water jacket through which temperature controlled water is re-circulated in order to perform the experiments at a desired temperature. The dialysis membrane is sandwiched between the two chambers and clamped in place tightly.

The donor chamber is filled with a known volume of and the permeation of solute through the membrane is monitored by periodic sampling of the

solution from the receptor chamber. The jacketed cell embodied is stirred throughout the study at 500 rpm employing a magnetic stirrer.

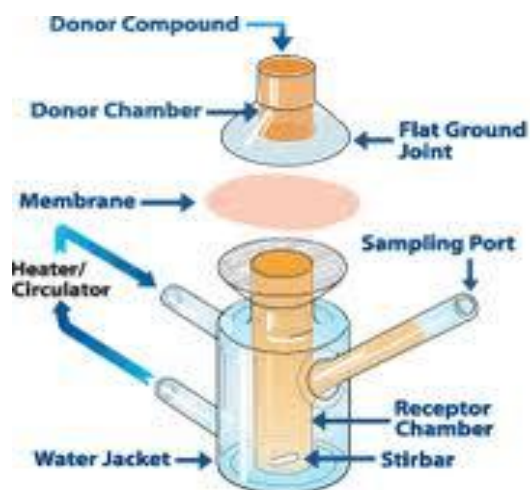


Figure: Franz diffusion cell

### Skin deposition studies

The amount of fluconazole retained in the skin was determined by skin deposition studies. At the end of permeation studies (8 hrs.), the skin was washed with methanol and homogenized for 5 min using an electric stirrer. The resulting solution was centrifuged at 7000 rpm for 10 min and supernatant was analyzed for drug content by UV-VIS double beam spectrophotometer at 260 nm.

### Drug release kinetics<sup>(94)</sup>

The release kinetic was studied by various kinetic models as zero order plot, first order plot, Higuchi plot and Korsmeyer-Peppas plot. To study the release kinetics of the ethosomal gel data obtained from in-vitro drug release studies were plotted in various kinetic models: zero order as cumulative amount of drug releases Vs. time, First order as log cumulative % of drug remaining Vs. time, Higuchi model as cumulative % of drug released Vs. square root of time and Korsmeyer-Peppas model as log cumulative % drug release Vs. log time. The best fit model was confirmed by the value of correlation coefficient near to 1.

### Zero-order model

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation:

$$Q_0 - Q_t = K_0 t \quad (1)$$

Rearrangement of equation (1) yields:

$$Q_t = Q_0 + K_0 t \quad (2)$$

Where  $Q_t$  is the amount of drug dissolved in time  $t$ ,  $Q_0$  is the initial amount of drug in the solution (most times,  $Q_0 = 0$ ) and  $K_0$  is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

*Application:* This relationship can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs in coated forms, osmotic systems, etc.

### First order model

This model has also been used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first order rate constant expressed by the equation:

$$\log C = \log C_0 - Kt / 2.303$$

Where  $C_0$  is the initial concentration of drug,  $k$  is the first order rate constant, and  $t$  is the time. The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of  $-K/2.303$ .

*Application:* This relationship can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices.

### Higuchi model

Graph was plotted between cumulative percentages of drug released vs. square root of time.

$$Q = K_{1/2} t^{1/2}$$

Where  $K$  is the constant reflecting the design variables of the system and  $t$  is the time in hours.

Hence, drug release rate is proportional to the reciprocal of the square root of time.

*Application:* this relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs.

### Korsmeyer-Peppas model<sup>(95)</sup>

Korsmeyer et al. (1983) derived a simple relationship which described drug release from a polymeric system. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model

$$M_t / M_\infty = Kt^n$$

Where  $M_t / M_\infty$  are a fraction of drug released at time  $t$ ,  $k$  is the release rate constant and  $n$  is the release exponent. The  $n$  value is used to characterize different release for cylindrical shaped matrices. For the case of cylindrical tablets,  $0.45 \leq n$  corresponds to a Fickian diffusion mechanism,  $0.45 < n < 0.89$  to non-Fickian transport,  $n = 0.89$  to Case II (relaxation) transport, and  $n > 0.89$  to super case II transport. To find out the exponent of  $n$  the portion of the release curve, where  $M_t / M_\infty < 0.6$  should only be used. To study the release kinetics, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time.

### Stability<sup>(96)</sup>

The stability study was carried out for ethosomal gel formulation. The most satisfactory formulation was sealed in a glass vial to a temperature of 40°C for 1 month, then at 25°C for 1 month, then at 40°C for 1 month. After this ethosomal gel was exposed to ambient room temperature and liquid exudates separating was

noted. At the end of 3 months, the samples were analyzed for physical characteristic study and the drug content.

## RESULTS AND DISCUSSION

### Preformulation studies Organoleptic properties

The following properties of drug were evaluated and results are obtained as:

Table: Organoleptic properties of fluconazole

Drug	Test	Specification	Observation
Fluconazole	Colour	White crystalline powder	White powder
Fluconazole	Odour	Odourless	Odourless

The observations noted were compared to the specifications given in the pharmacopoeia to confirm the identity of the drug and it was found that observations noted complied with the specifications.

### Solubility studies

Solubility studies are performed to determine the solubility of drug in different solvents. The solubility is expressed in terms of ratio of solute and solvent. Fluconazole was found to be soluble in methanol, acetone, 0.1 N HCL, ethanol, distilled water, hexane, PBS of pH 5.5, 6.8, 7.4, chloroform, ether and propylene glycol.



Table: Solubility profile of fluconazole

S.NO.	Quantity of drug	Solvent	Quantity of solvent	Inference
1.	50 mg	Methanol	5 ml	Freely soluble
2.	50 mg	Acetone	5 ml	Soluble
3.	50 mg	0.1 N HCL	5 ml	Sparingly soluble
4.	50 mg	Ethanol	5 ml	Sparingly soluble
5.	50 mg	Distilled water	5 ml	Slightly soluble
6.	50 mg	Hexane	5 ml	Very slightly soluble
7.	50 mg	PBS pH 5.5	5 ml	Slightly soluble
8.	50 mg	PBS pH 6.8	5 ml	Slightly soluble
9.	50 mg	PBS pH 7.4	5 ml	Slightly soluble
10.	50 mg	Chloroform	5 ml	Sparingly soluble
11.	50 mg	Ether	5 ml	Slightly soluble
12.	50 mg	Propylene glycol	5 ml	Slightly soluble

### Melting point

Melting point of fluconazole was found to be 138 °C. Melting point was measured three times and mean was noted. A sharp transition took place from solid to liquid at 138 °C, indicating that the sample was pure and free from impurities.

### Partition co-efficient

Partition co-efficient was measured three times and mean was noted. Hence partition coefficient was found to be 1.01± 0.01.

### Determination of pH

The pH was measured three times and mean was noted. Hence pH of fluconazole was found to be 6.8.

**FTIR analysis:** - FTIR spectroscopic analysis was carried out to characterize drug. The FTIR spectra obtained was compared with that given in pharmacopoeia for fluconazole. Diagnostic peaks and finger print regions were found identical. These characteristics peaks are useful in identification of drug. FTIR of carbopol 934 and mixture containing fluconazole and carbopol 934 was done for drug compatibility studies. The results obtained showed that there occur no interactions between the components when taken together.

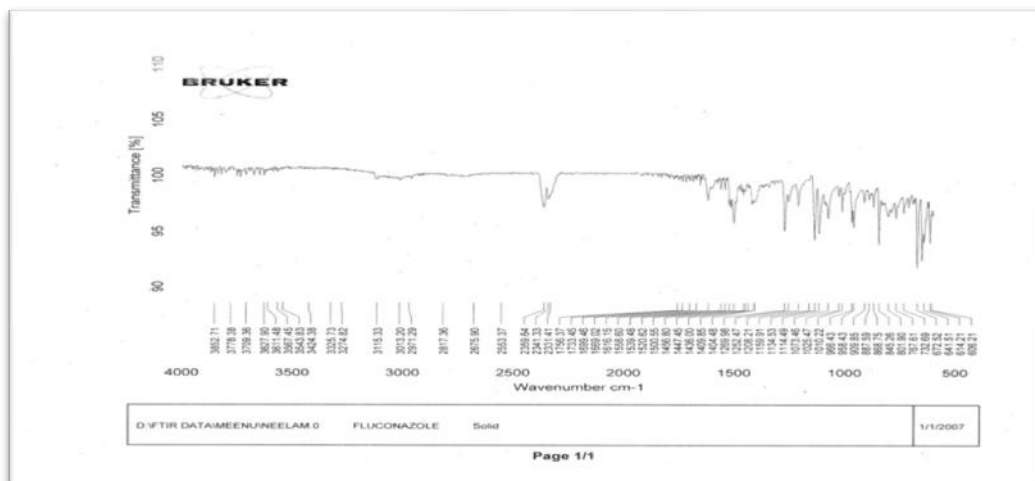


Figure: FTIR of Fluconazole

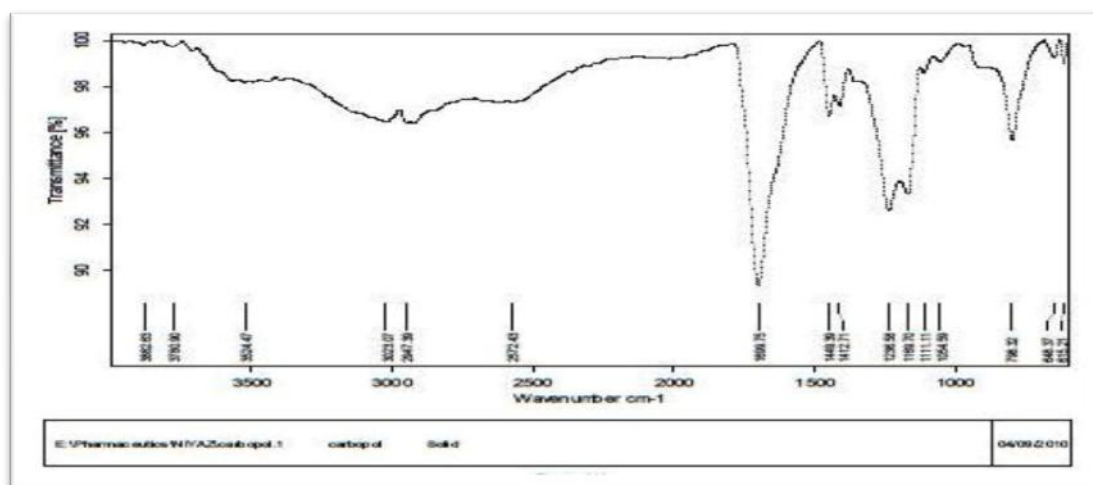


Figure: FTIR of Carbopol 934

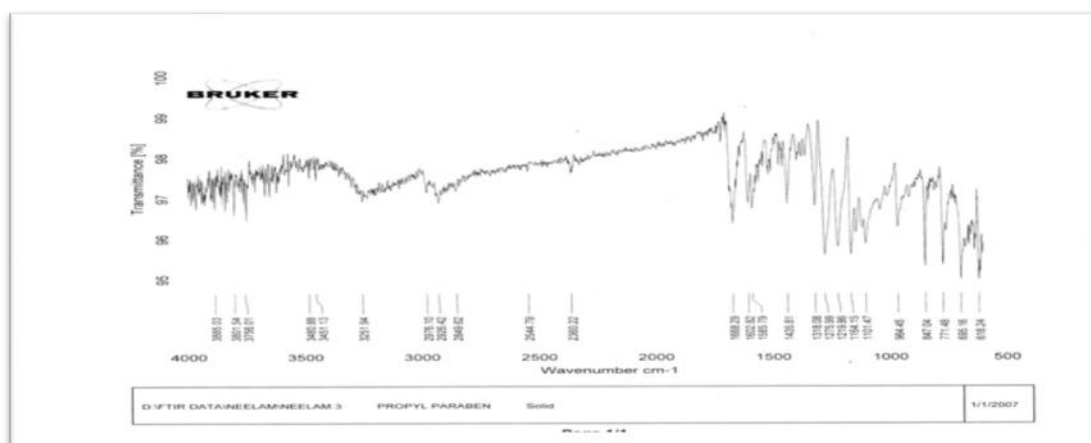


Figure: FTIR of Propyl paraben

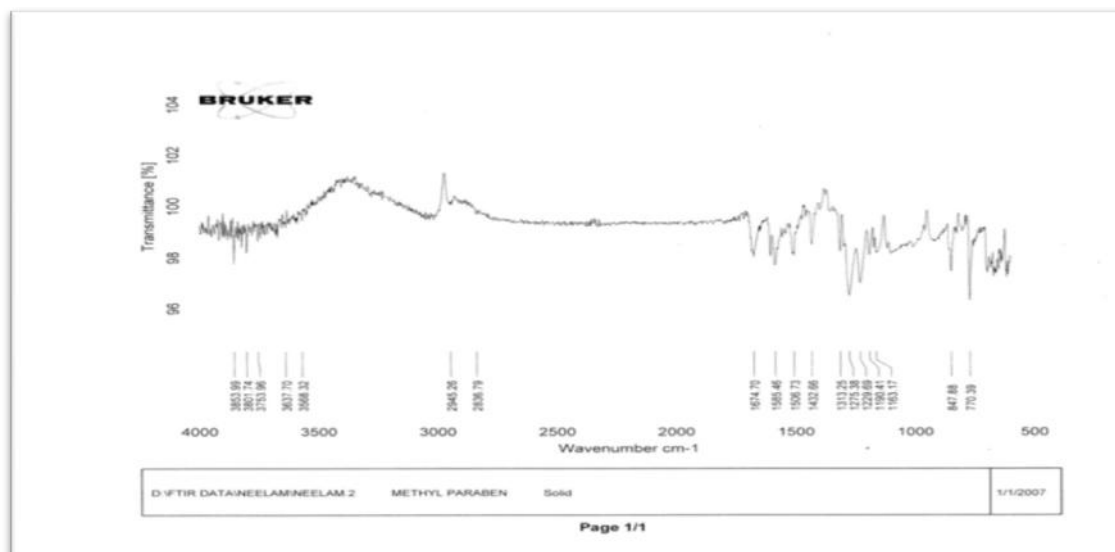


Figure: FTIR of Methyl paraben

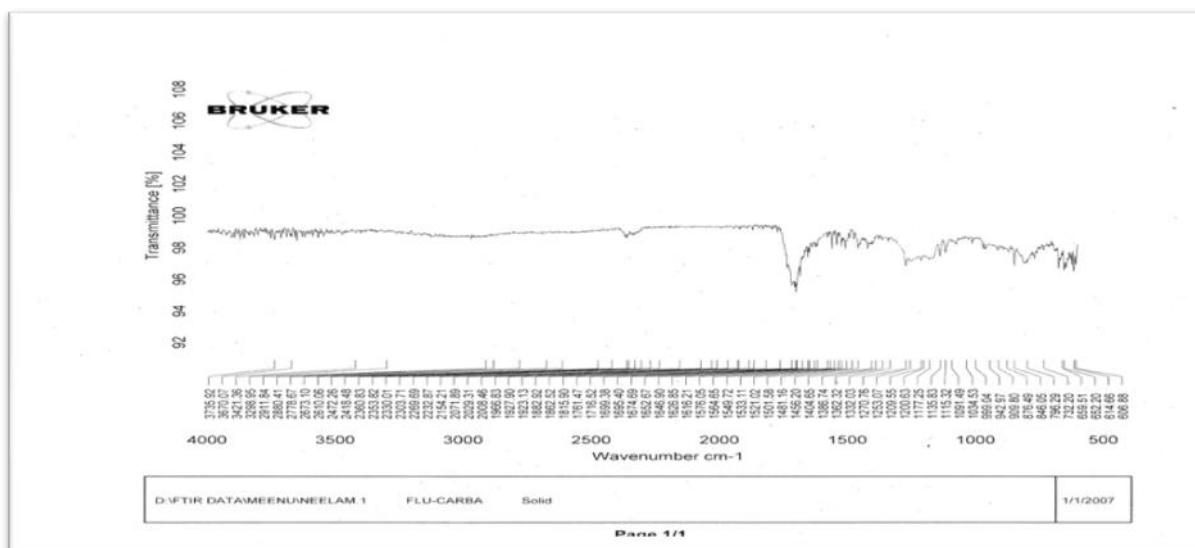


Figure: FTIR of mixture containing fluconazole, carbopol, methyl paraben and propyl paraben.

All the group were present at same value hence drug sample was genuine and free from any major type of impurities.

Table: Comparison between peaks obtained in drug and in a mixture

Peak obtained in drug (frequency $\text{cm}^{-1}$ )	Description	Peak obtained in mixture (frequency $\text{cm}^{-1}$ )
3424.38	OH Stretching	3421.36
2817.36	CH <sub>2</sub> Stretching	2880.41
3013.20	CH (Aromatic Stretching)	3298.95
1616.15	C = N Stretch	1616.21
1456.80	CH (Aromatic bending)	1456.20
868.75	C - F Stretch	876.49

### Analysis by UV- Visible spectrophotometry

#### Preparation of standard graph

**Stock solution of Fluconazole:** Stock solution of 100 $\mu\text{g}/\text{ml}$  was prepared by dissolving 10 mg of fluconazole in 100 ml of methanol. Dilution in the range of 10 to 100  $\mu\text{g}/\text{ml}$  were scanned for determining  $\lambda_{\text{max}}$  from 200-400 through UV spectrophotometer and  $\lambda_{\text{max}}$  was found to be at 260 nm for fluconazole.

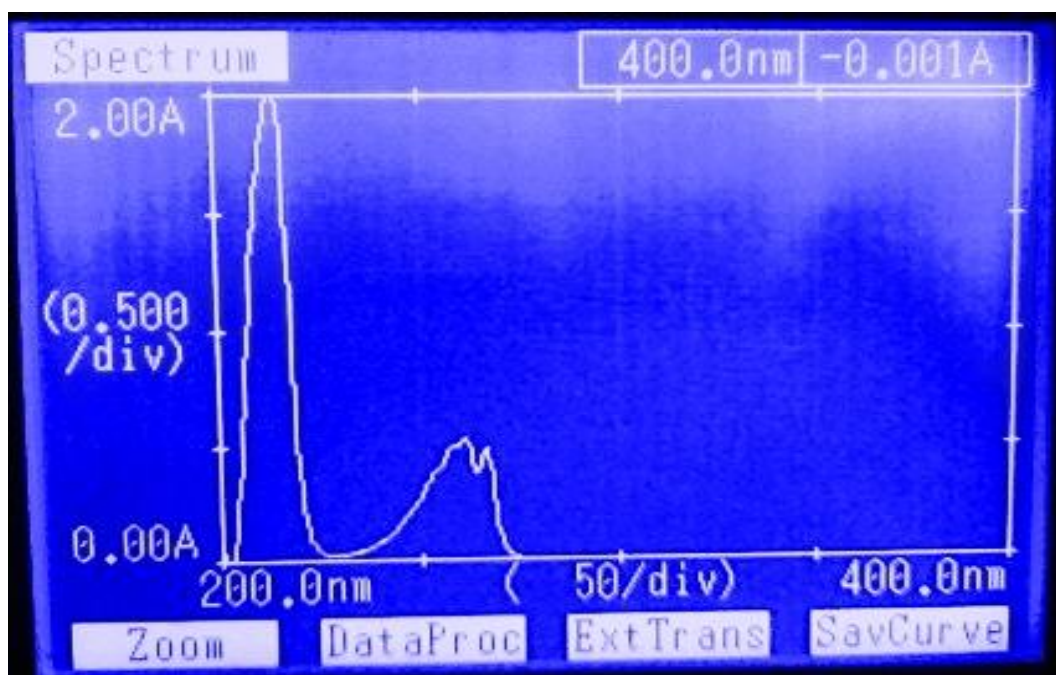


Figure:  $\lambda_{\text{max}}$  of Fluconazole in methanol

Table: Absorbance of different dilutions of drug at 260 nm in methanol

S.NO.	Concentration (µg/ml)	Absorbance (mean ± SD)(n=3)
1.	0	0
2.	10	0.131 ± 0.001
3.	20	0.262 ± 0.004
4.	30	0.355 ± 0.002
5.	40	0.448 ± 0.003
6.	50	0.540 ± 0.005
7.	60	0.633 ± 0.002
8.	70	0.728 ± 0.001
9.	80	0.823 ± 0.001
10.	90	0.911 ± 0.005
11.	100	0.999 ± 0.003

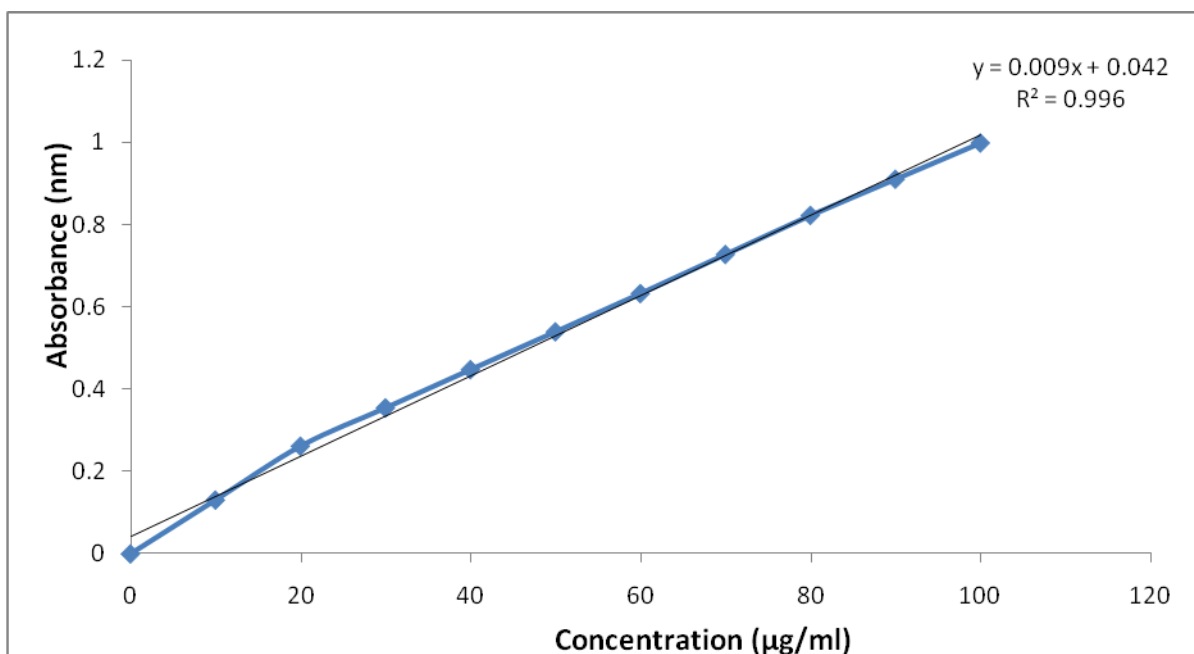


Figure: Standard calibration curve of fluconazole at 260nm in methanol

**Preparation of calibration curve in PBS 7.4:** since PBS (7.4) is taken as dissolution medium for ethosomal gel, the standard curve was obtained to calculate the concentration of unknown samples for dissolution studies and drug content.

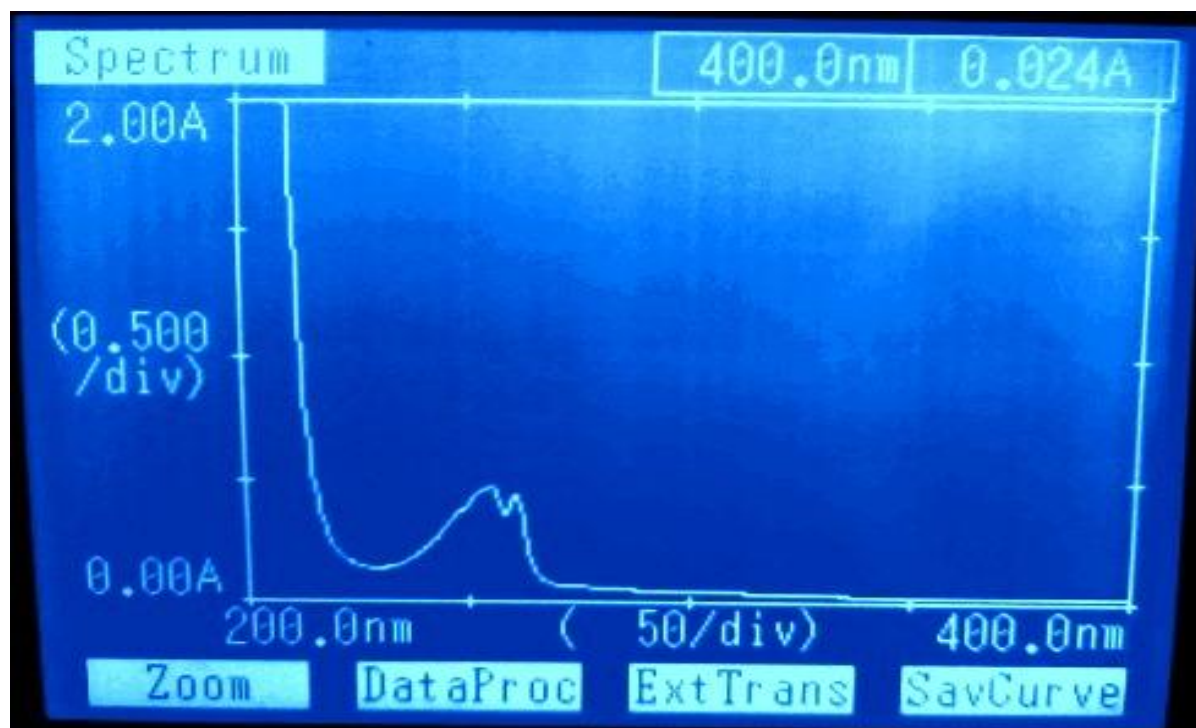


Figure:  $\lambda_{max}$  of Fluconazole in P.B.S. pH 7.4

Table: Absorbance of different dilutions of drug at 260 nm in PBS pH 7.4

S.NO.	Concentration ( $\mu\text{g/ml}$ )	Absorbance (mean $\pm$ SD)(n=3)
1.	0	0
2.	10	0.119 $\pm$ 0.000
3.	20	0.172 $\pm$ 0.001
4.	30	0.215 $\pm$ 0.001
5.	40	0.268 $\pm$ 0.002
6.	50	0.318 $\pm$ 0.002
7.	60	0.372 $\pm$ 0.000
8.	70	0.438 $\pm$ 0.004
9.	80	0.482 $\pm$ 0.003
10.	90	0.523 $\pm$ 0.003

11.	100	0.577 ± 0.004
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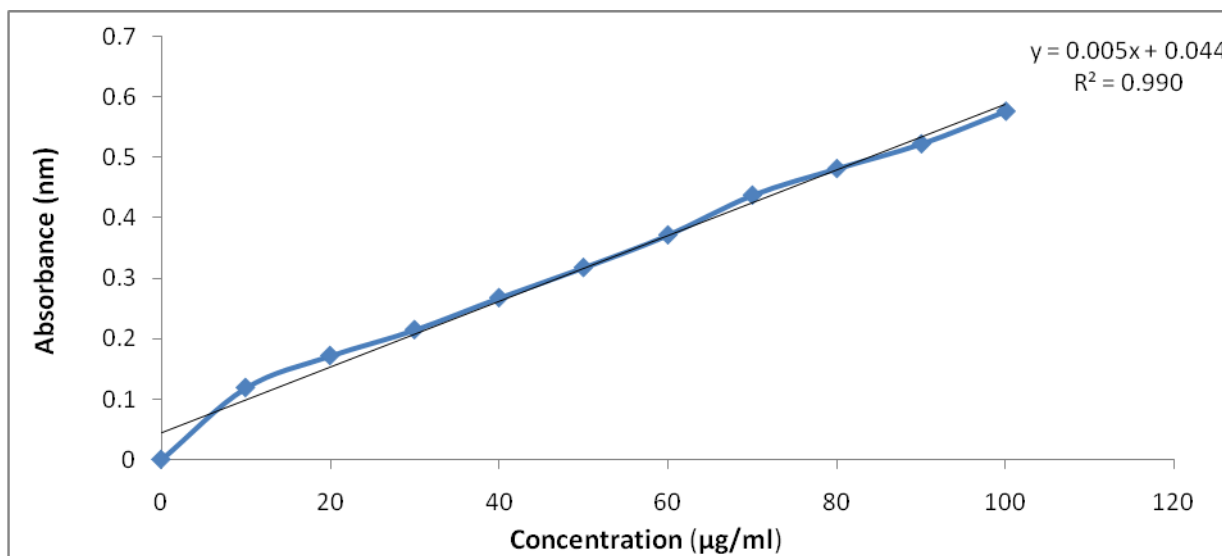


Figure: Standard calibration curve of fluconazole at 260nm in P.B.S pH 7.4

### EVALUATION OF ETHOSOMES

Drug entrapment efficiency

Drug entrapment efficiency was calculated as by formula:

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of entrapped drug recovered}}{\text{Total amount of drug}} \times 100$$

Table: Effect of different ratio of phospholipids and ethanol on drug entrapment

S.No.	Formulation code	Entrapment efficiency
1.	F 1	38%
2.	F 2	46%
3.	F 3	58%
4.	F 4	65%
5.	F 5	72%
6.	F 6	78%
7.	F 7	68%
8.	F8	90%
9.	F 9	62%

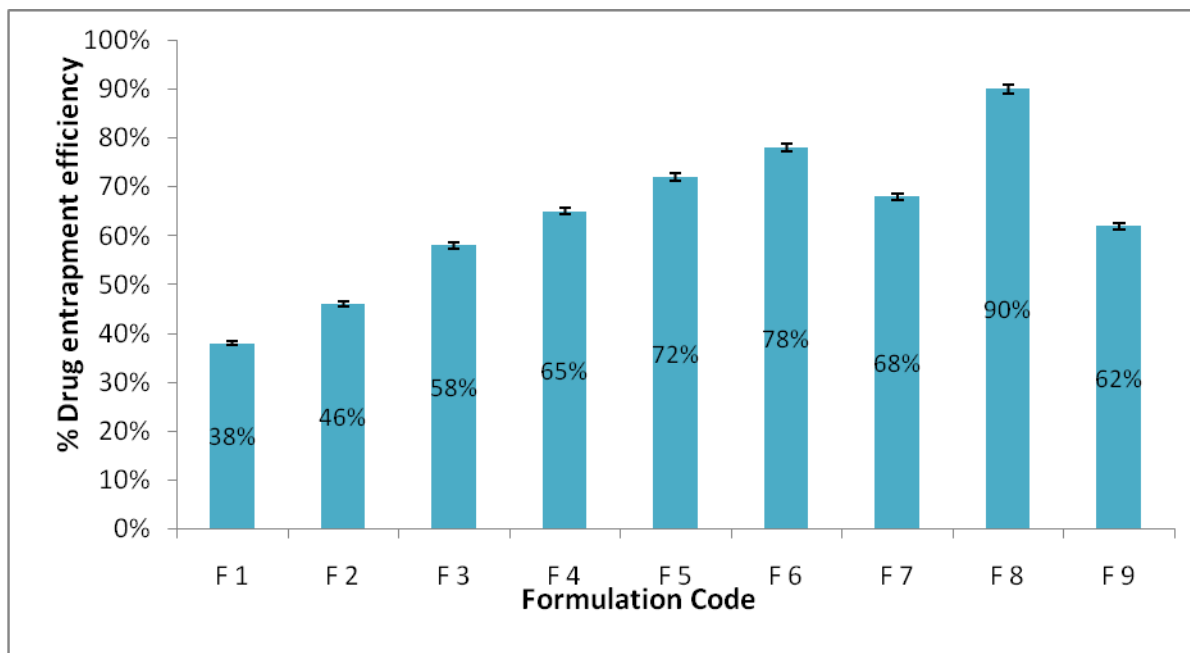


Figure: Drug entrapment efficiency of fluconazole ethosomes

Drug entrapment efficiency of different formulations was calculated. Drug entrapment efficiency of formulation F8 was found to be highest. So, this formulation was selected for further ethosomal formulation.

**Transmission electron microscopy(TEM):**F8 formulation was selected as best formulation and therefore subjected for TEM to obtain the picture of ethosomes on scale bar of 200nm with magnification 13.0×4000 as shown below. On characterization spherical, unilamellar vesicles with smooth surface were observed under transmission electron microscopy (TEM).

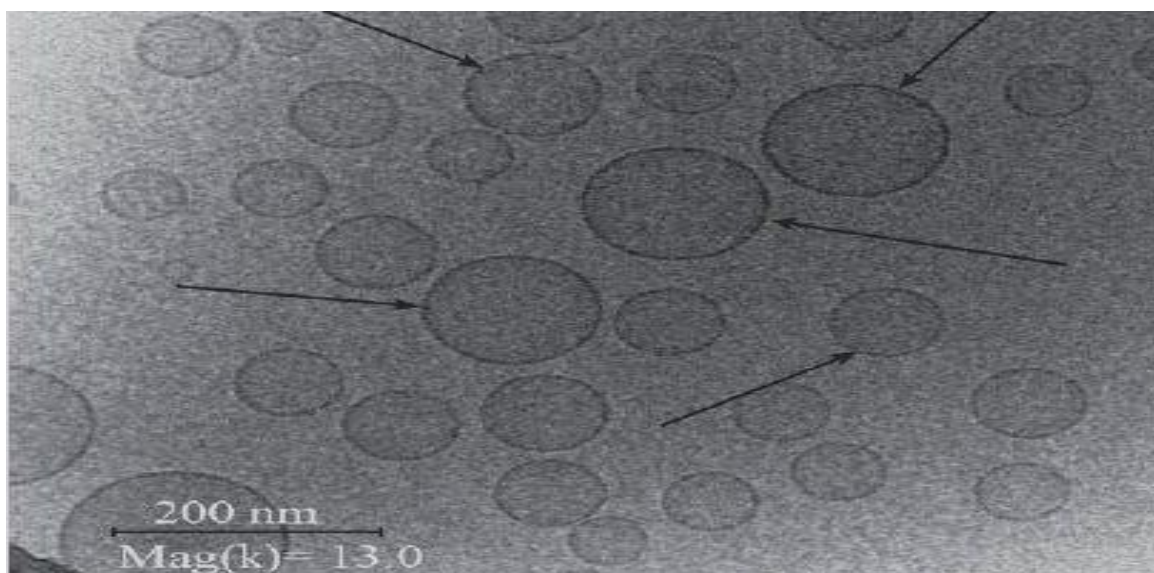


Figure: TEM of F8 formulation



### Zeta potential measurement

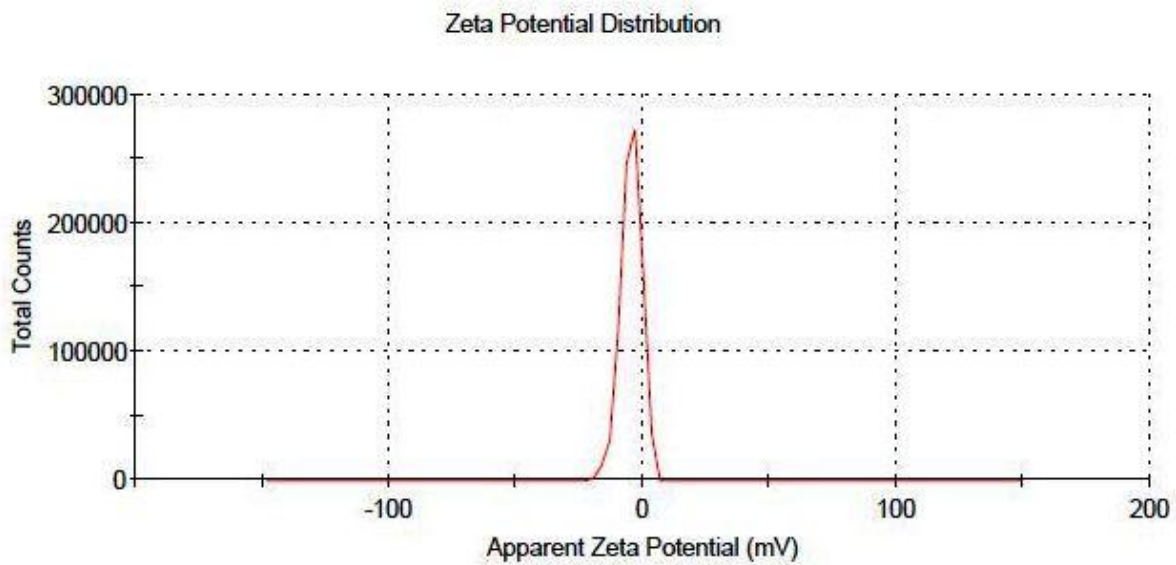


Figure:

### Zeta potential of F8 formulation

Zeta potential of F8 formulation was found to be - 4.61 mv.

### Particle size measurement of F8 formulation

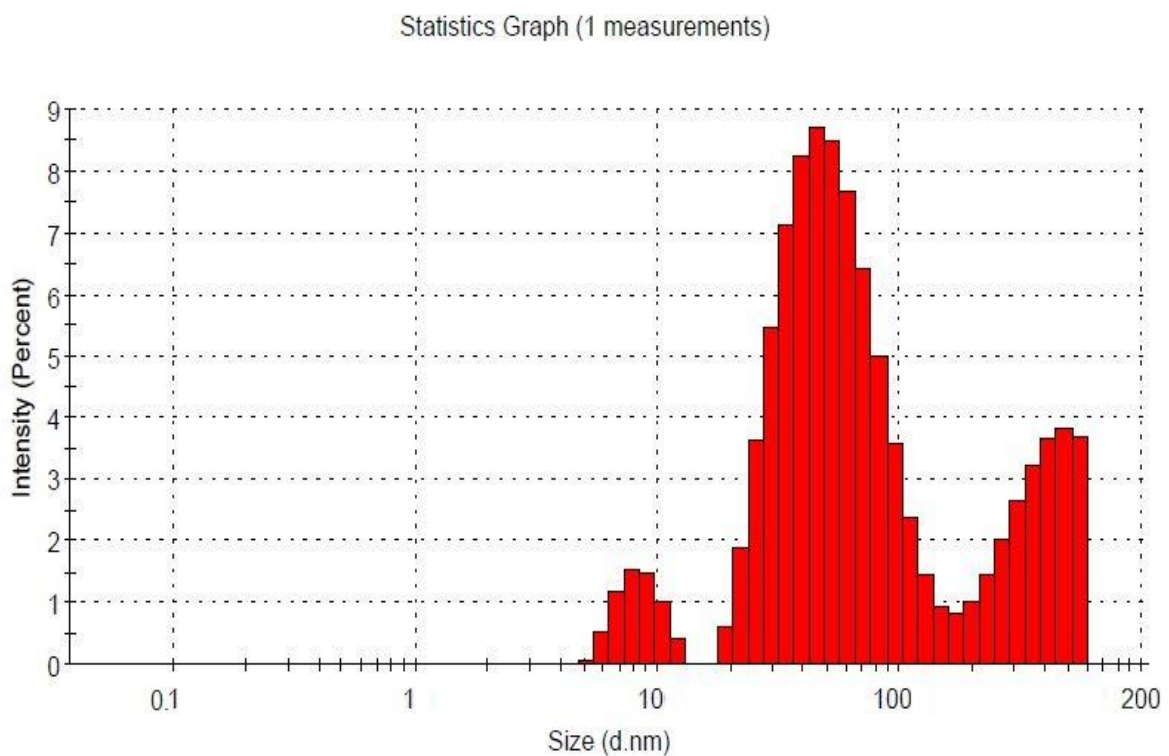


Figure: Particle size of F8 formulation

Vesicular size of ethosomes of F8 formulation was in the range of 5 to 200 nm.

## PHYSICAL EVALUATION OF ETHOSOMAL GEL

The ethosomal gel formulation of fluconazole was evaluated for

### Organoleptic characteristics

**Colour** = pale (yellow) to colourless

**Odour** = characteristic

**Appearance** = translucent

**Phase separation** = no

**Occlusiveness** = yes

**Washability** = washable

### Determination of pH of gel base and ethosomal gel

The pH of gel base and freshly prepared F8 ethosomal gel was found to be 7.5 and 8.0 respectively.

### Viscosity

The viscosity of carbopol 934 gel base and ethosomal gel by brookfield viscometer was found to be 744,00 and 73,200 cps (centipoise).

### Spreadability

The spreadability of ethosomal gel was found to be  $14.79 \text{ g.cm}^2$ . The spreadability results showed that ethosomal gel was most effective i.e. it showed best result for spreadability.

### Extrudability study

The extrudability of ethosomal gel was found to be positive.

### Percentage yield

The % yield of ethosomal gel was found to be 97.18 %.

### Homogeneity and grittiness

Ethosomal gel was found to be homogeneous and no grittiness was noted.

### Skin irritation study

The results of skin irritation study revealed no irritation from ethosomal gel.

**In vitro release study** *In vitro* release study was performed to determine amount of drug released at different interval of time.



Figure: Franz diffusion cell with skin mounted between compartments.

Table: Release of drug from F8 formulation and marketed formulation.

Time (min.)	% cumulative release of F8 formulation in 7.4 pH PBS	% cumulative release of marketed fluconazole gel in 7.4 PBS
0	0	0
15	14.16 ± 0.12	8.52 ± 0.02
30	19.84 ± 0.15	12.08 ± 0.06
45	36.02 ± 0.10	17.56 ± 0.20
60	37.98 ± 0.16	19.02 ± 0.09
120	42.54 ± 0.18	25.66 ± 0.31
180	46.09 ± 0.17	27.03 ± 0.38
240	59.76 ± 0.11	36.78 ± 0.49
300	63.34 ± 0.19	42.08 ± 0.15
360	70.03 ± 0.20	50.98 ± 0.18
420	82.72 ± 0.13	61.78 ± 0.42
480	92.00 ± 0.79	70.00 ± 0.22

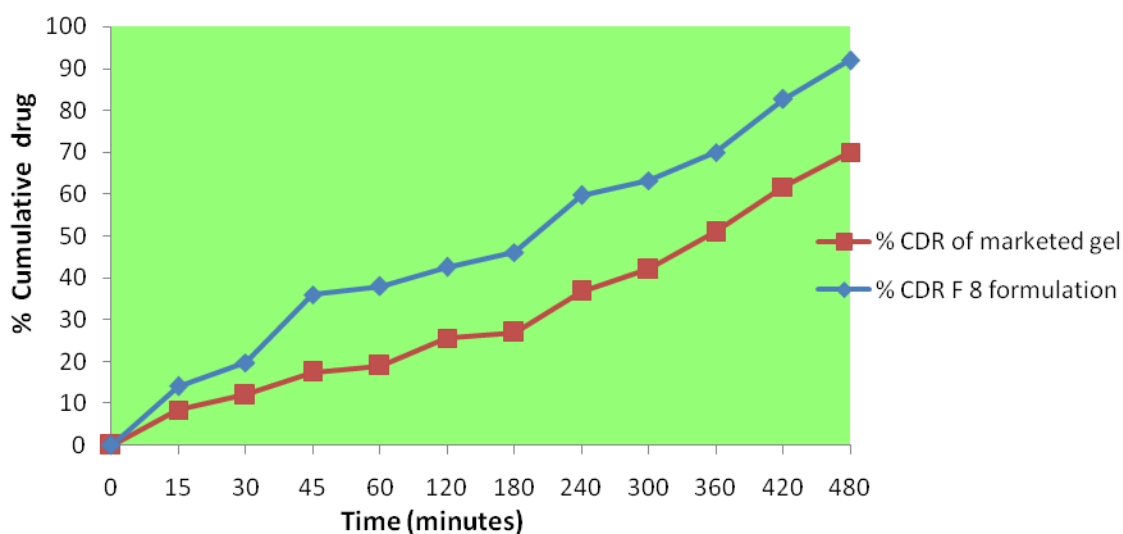


Figure: Rate release of drug from F8 formulation and marketed gel in P.B.S. pH 7.4

Rate of release from different formulation are given in graph and table. It may be observed that release of drug occurred fast initially then after some time release become slower. From graph it was observed that cumulative release of fluconazole was more from ethosomal formulation than marketed

fluconazole gel over a period of 8 hrs. Ethosomal formulation was designed to achieve high permeability and ultimately increase the bioavailability of the drug.

### KINETICS OF DRUG RELEASE

The release kinetic of F8 formulation in phosphate buffer saline pH 7.4 was studied by various kinetic models. The following data was obtained.

Table: Drug release data of F8 formulation in P.B.S. pH 7.4

Time (minutes)	Log time	Square root of time	% cumulative release of F8	Log % cumulative release of F8	% cumulative remaining	Log % cumulative remaining
0	0	0	0	0	100	2
15	1.18	3.87	14.16	1.151	85.84	1.933
30	1.48	5.48	19.84	1.297	80.16	1.903
45	1.65	6.71	36.02	1.556	63.98	1.806
60	1.75	7.75	37.98	1.579	62.02	1.792
120	2.08	10.95	42.54	1.628	57.46	1.759
180	2.26	13.42	46.09	1.663	53.91	1.731
240	2.38	15.49	59.76	1.776	40.24	1.604
300	2.48	17.32	63.34	1.801	36.66	1.564
360	2.56	18.97	70.03	1.845	29.97	1.476
420	2.62	20.49	82.72	1.917	17.28	1.237
480	2.68	21.91	92.00	1.971	6.36	0.803

### ZERO ORDER PLOT

Graph was plotted between % cumulative drug release Vs time .

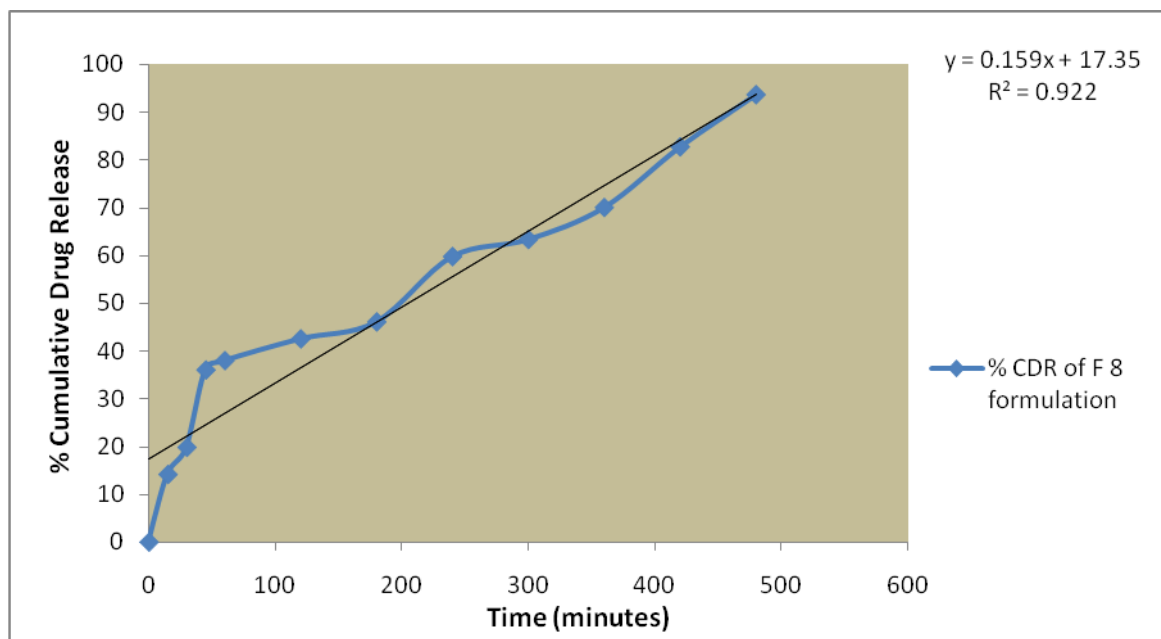


Figure: Zero order plot for drug release kinetics of F8 formulation in P.B.S. pH 7.4

### FIRST ORDER MODEL

Graph was plotted between log % cumulative drug remaining Vs time .

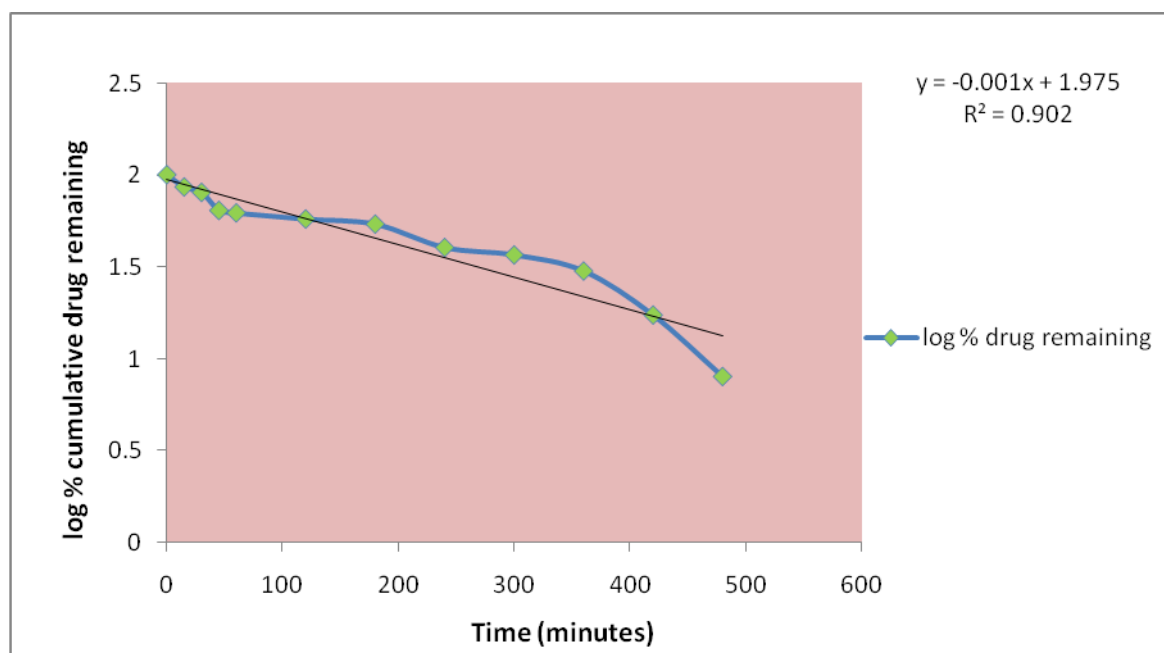


Figure: First order plot for drug release kinetics of F8 formulation in P.B.S. pH 7.4

### HIGUCHI'S MODEL

Graph was plotted between % cumulative drug release Vs square root of time.

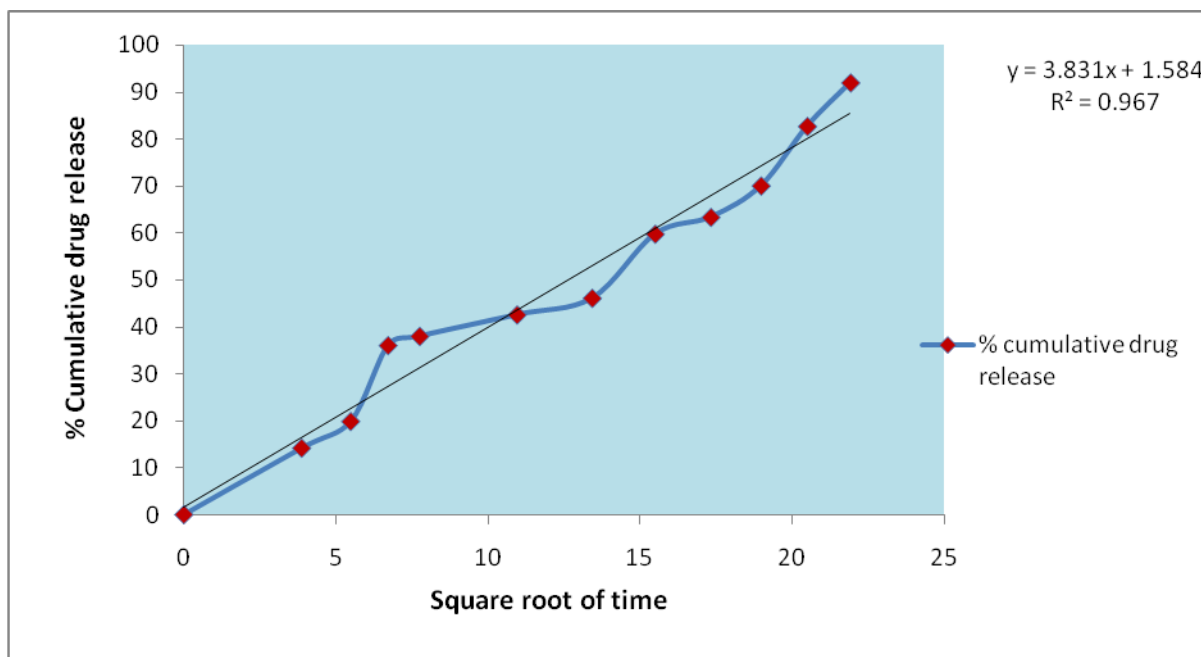


Figure: Higuchi plot for drug release kinetics of F8 formulation in P.B.S. pH7.4

### KORSMEYER-PEPPAS MODEL

Graph was plotted log % cumulative drug released Vs log time.

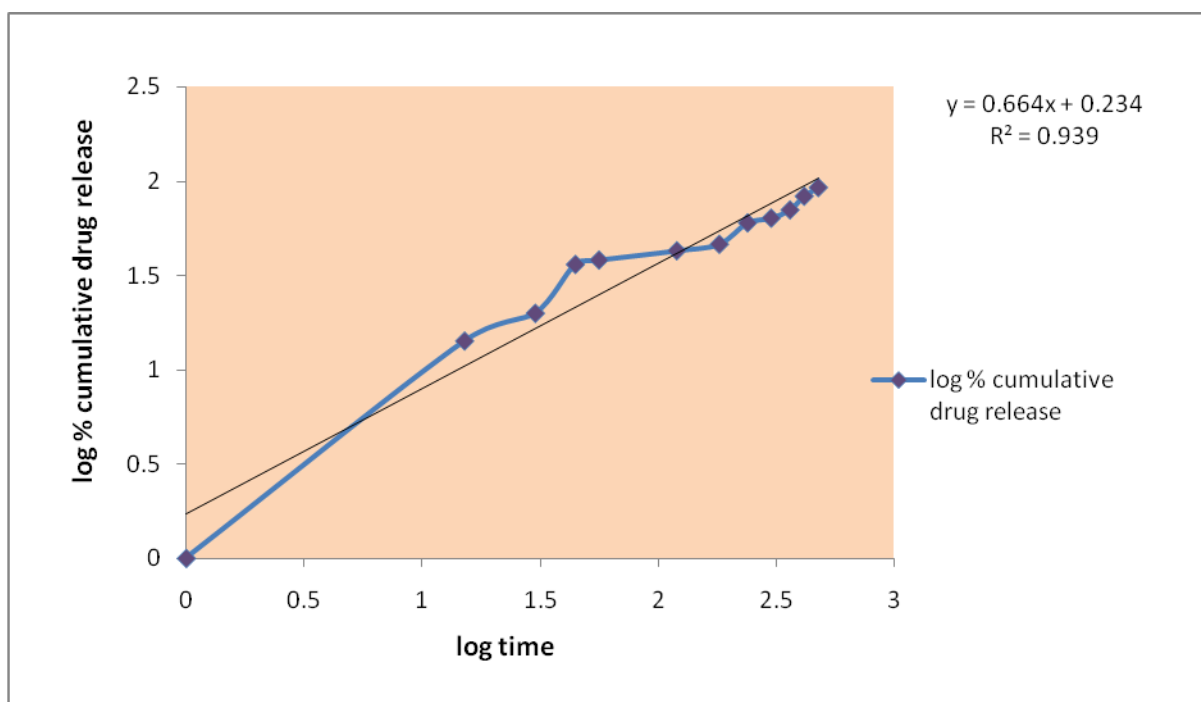


Figure: Peppas plot for drug release kinetics of F8 formulation in P.B.S. pH 7.4

Table: Kinetics of drug release of F8 Formulation in P.B.S. pH 7.4.

Formulation code	Zero order		First order		Higuchi		Korsmeyer Peppas	
	R <sup>2</sup>	K <sub>o</sub>	R <sup>2</sup>	K <sub>f</sub>	R <sup>2</sup>	K <sub>h</sub>	R <sup>2</sup>	N
F8 formulation	0.9222	0.159	0.9027	0.0018	0.9678	3.8316	0.9393	0.6647

The data obtained for *in vitro* release were fitted into equation for the Zero order, First order, Higuchi and Korsmeyer peppas release models. The interpretation of data was based on the value of the resulting regression coefficients.

From these values, it was observed that the Higuchi model was found to be best suited with R<sup>2</sup> value of 0.9678. Higuchi model be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some topical systems.

### Stability Studies

Stability studies performed at 40°C±1°C, 25°C±1°C for 3 months showed good storage stability.

### Physical appearance

It was observed that optimized gel kept for 3 months under 40°C ± 1°C as well as 25°C ± 1°C temperature conditions showed no change in their physical appearance.

### Phase separation

No phase separation was observed in the optimized ethosomal gel.

### Content uniformity

Optimized ethosomal gel kept for 3 months under 40°C±1°C as well as 25°C±1°C temperature conditions were studied for uniformity of content. The results showed no significant changes in

content uniformity at 40°C±1°C after 2 months. At 40°C±1°C content uniformity was found to show approximately (92±0.79% to 89±0.12%) decrease and at 25°C±1°C content uniformity decreased from (92±0.79% to 78.90±0.91%).

### CONCLUSION

The present work on the preparation of topical ethosomal gel containing fluconazole is an attempt to utilize the immense potential of ethosomes as a carrier to increase the permeability. In this contribution, we developed and evaluated the ethosomes containing fluconazole to obtain the optimized formulation which suit for application as skin delivery system.

Fluconazole is used for the treatment of local and systemic fungal infection. But one of the major problems for efficient drug delivery is low penetration rate of fluconazole due to its high solubility and low permeability.

Further, the physicochemical modification in the drug by means of phospholipid membrane also promises to prolong the drug action. A number of problems associated with drug molecules such as bioavailability, degradation, stability and side effects can be overcome by incorporating it into ethosomes.

The ethosomes of fluconazole were prepared by cold method and evaluated. *In vitro* release of F8 formulation was higher than the marketed gel (Flucos gel). Based on  $R^2$  value the F8 formulation followed Higuchi model for the mechanism of drug release.

**So, the major objectives have been achieved successfully:**

Preparation and evaluation of ethosomal gel of fluconazole by cold method.

To study the effect of different concentrations of phospholipid and ethanol on drug entrapment efficiency to obtain an optimized formulation, calculate the % drug release and study kinetic model complying with the formulation.

To deliver the drug in non-toxic, biodegradable form topically at or near the site of application.

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