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Formulation of SDS Aided and Ethosomal Fluconazole Topical Gel for Improved Antifungal Effect

Aniket Chouksey[1], Dr. B.K. Dubey[2], Dr. Deepak Kumar Basediya[3], Vinay Mahajan[4]

[1]PG Scolar, Technocrates Institute of Technology pharmacy Bhopal M.P. India
[2]Principal, Technocrates Institute of Technology pharmacy Bhopal M.P. India
[3]Associate Professor& HOD, Technocrates Institute of Technology pharmacy Bhopal M.P. India
[4]Assistent Professor at Dr. Apj Abdul Kalam University Indore Madhya Pradesh India

Abstract

Fungal infections are the leading cause of death in both advanced and developing countries. This is due to the use of immunosuppressive treatments, long term use of antibiotics, and longer survival of immuno compromised individuals. Fluconazole, commonly known as *Diflucan*, is an antifungal drug used for the treatment of both systemic and superficial fungal infections in a variety of tissues. This drug is an *azole* antifungal, in the same drug family as ketoconazole and itraconazole. Fluconazole has many advantages over the other antifungal drugs including the option of oral administration [43]. This research was designed to formulate & evaluate different formulation of a topical gel containing fluconazole by using a polymer with different concentration as Carbopol 934p & Di potassium Hydrogen Orthophosphate. Methanol was used as a penetration enhancer. The evaluation of formulated fluconazole topical gel was carried out for a physical appearance, pH-value, spreadability, homogeneity, drug content. The formulated gel showed good physical characteristics. formulation EF2 select as a optimized formulation because of its good Spreadability (11.15±0.32), Viscosity (3310±25) and pH (6.80±0.35). The maximum % assay was also found in formulation F2 (99.45±0.25).

Keywords Formulation, SDS, Fluconazole, Topical Gel, Antifungal Effect

Introduction

Fungal infections are the leading cause of death in both advanced and developing countries. This is due to the use of immunosuppressive treatments, long term use of antibiotics, and longer survival of immuno compromised individuals [1].

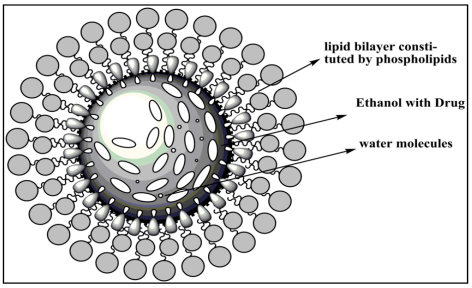

Figure 1: Structure of Ethosomes

Ethosomes are ethanolic liposomes. Ethosomes (Fig 1) can be defined as non- invasive delivery carriers that enable drugs to reach deep into the skin layers or the systemic circulation. They are soft and flexible nanovesicles.

Potential advantage of ethosomal drug delivery system

- Delivery of large molecules (peptides, protein molecules) is possible.
- It contains non-toxic raw material in formulation.
- Enhanced permeation of drug through skin for transdermal drug delivery.
- Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
- High patient compliance: The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.

Disadvantages of ethosomal drug delivery

- Drugs that require high blood levels cannot be administered –limited to onlypotent drugs (daily dose -10mg or less)
- Poor practical yield.
- Ethosomes with poor shells may clump together and leads to precipitation.
- Transfer of ethosomes from organic to aqueous layer leads to loss of product
- Ethosomal administration is not a means to achieve rapid bolus type druginput, rather it usually designed to offer slow, sustained drug delivery.

Composition of Ethosomes

Ethosomes exhibit lipid bilayer like liposomes; however, they differ from liposomes in terms of composition (high content of ethanol). The ethosomes are composed of hydroalcoholic or hydro/glycolicphospholipid in which the concentration of alcohol is relatively high. Ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine, phosphatidic acid, phosphatidylserine, Phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl inositol, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). Somepreferred phospholipids such as Phospholipon 90 (PL-90).

Methods of Preparation

Classic method

The phospholipid and drug are dissolved in ethanol and heated to 30°C±1°C in a water bath. Double distilled water is added in a fine stream to the lipid mixture, withconstant stirring at 700rpm, in a closed vessel. The resulting vesicle suspension is homogenized by passing through a polycarbonate membrane using a hand extruderfor three cycles [21].

Mechanical dispersion method

Soya phosphotidylcholine is dissolved in a mixture of chloroform: methanol in round bottom flask (RBF). The organic solvents are removed using rotary vacuum evaporator above lipid transition temperature to form a thin lipid film on wall of the RBF. Finally, traces of solvent mixture are removed from the deposited lipid film by leaving the contents under vacuum overnight. Hydration is done with different concentration of hydroethanolic mixture containing drug by rotating the RBF at suitable temperature.

Cold method

In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 300C in a water bath. The water heated to 300C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of ethosomal formulation canbe decreased to desire extend using sonication [22] or extrusion [23] method.

Hot method

In this method phospholipid is dispersed in water by heating in a water bath at 400Cuntil a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 400C. Once both mixtures reach 400C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties. The vesicle size of ethosomalformulation can be decreased to the desire extent using probe sonication.

Mechanism of drug penetration

Ethanol effect

Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density oflipid multilayer of cell membrane.

Ethosomes effect

Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin [25].

Material and Method

Materials

Materials was used in the investigation are listed in Table 1.

Table 1: Materials used for formulation development of ethosomal gel

Sr. No.	Chemicals	Supplier
1.	Fluconazole	Gift sample obtained from bioplus lifescience Bangalore
2.	Lecithin	Hi media Mumbai
3.	Disodium Hydrogen Phosphate	S. D. Fine Chem. Ltd., Mumbai
4.	DipotassiumHydrogenOrthophosphate	S. D. Fine Chem. Ltd., Mumbai
5.	Sodium Chloride	S. D. Fine Chem. Ltd., Mumbai
6.	Methanol	Qualigens Fine Chemicals, Mumbai
7.	Ethanol	Qualigens Fine Chemicals, Mumbai
8.	Chloroform	Qualigens Fine Chemicals, Mumbai
9.	Carbopol 934p	S. D. Fine Chem. Ltd., Mumbai
10.	Methyl Paraben	S. D. Fine Chem. Ltd., Mumbai
11.	Propyl Paraben	S. D. Fine Chem. Ltd., Mumbai
12.	Propylene Glycol	S. D. Fine Chem. Ltd., Mumbai

Instruments

Table 2: Instruments used for the preparation and evaluation of ethosomalgel

Sr. No.	Instruments	Supplier
1.	UV -Visible Spectrophotometer	Labindia 3000+
2.	Fourier Transform Infra Red Spectroscopy	Brucker, Germany
3.	Mechanical Stirrer	Bionics Scientifics, Delhi
4.	Optical Microscope	Lyzer, Ambala
5.	Micro Centrifuge	REMI laboratory, Mumbai
6.	Franz Diffusion Cell	Electro Lab, Mumbai
7.	pH Meter	Accumax India, New Delhi
8.	Electronic Balance	Contech Instruments Ltd. , Mumbai
9.	Melting Point Apparatus	Contech Instruments Ltd. , Mumbai

10.	Hot Air Oven	Oracle Equipments, New Delhi
11.	Vortex Apparatus	Ambros Lab Equipments, Ambala
12.	Brook Field Viscometer	Precision Electro Instrumentation India Private Limited, Thane
13.	Differential ScanningCalorimeter	Perkin-Elmer India Pvt. Ltd., Thane
14.	Rotary Vaccum Evaporator	Microtech Scientific Instruments, NewDelhi
15.	IR Moisture Balance	Scope Enterprises, New Delhi
16.	Transmission ElectronMicroscope	Hitachi, Tokyo, Japan
17.	Zeta Sizer	Malvern Instruments, UK
18.	Sonicator	Athena Technology, Thane

Preparation of Ethosomes of Fluconazole

Soya PC (0.5 to 1.5% w/v) was dissolved in ethanol (10-20% v/v) and heated up to30 ± 1°C in a water bath in a closed vessel [50]. Distilled water or drug solution in distilled water (0.5% w/v solution) containing SDS (50mg), which is previously heated up to 30 ± 1°C, was added slowly in a fine stream to the above ethanolic lipidsolution with continuous mixing using a magnetic stirrer at 900 rpm. Mixing was continued for another 5 minutes and finally, the vesicular dispersions resulted was left to cool at room temperature (25 ± 1°C) for 45 minutes.

Results and Discussion

Characterization of Fluconazole

Physical evaluation

Table 2: Physical evaluation of drug

S. No.	Sensory characters	Physical evaluation of Fluconazole
1.	Colour	White to off white powder
2.	Odor	Odorless
3.	Taste	Tasteless

Solubility: Solubility of the drug was determined by taking some quantity of drug (about 1-2 mg) in the test tube separately and added the 5 ml of the solvent (water, ethanol, methanol, 0.1 N HCl, 0.1 N NaOH, 7.2 pH phosphate buffer and Chloroform) Shake vigorously and kept for some time. Note the solubility of the drug in various solvents (at room temperature) [46].

Table 3: I.P. Ranges for Solubility

Descriptive term	Parts of solvent required for Parts of solute
Very soluble	Less than 1
Freely soluble	From 1to 10
Soluble	From 10 to 30
Sparingly soluble	From 30to 100
slightly soluble	From 100 to1000
Very slightly soluble	From 1000 to 10000
Practically insoluble	10000 or more

Table 4: Solubility of Fluconazole

Solvent used	Results of Solubility
Distilled Water	Insoluble
0.1 N Hydrochloric acid	Soluble
Ethanol	Soluble
Methanol	Soluble
Chloroform	Insoluble
0.1 N NaOH	Insoluble
7.2 pH phosphate buffer	Soluble

Melting point

A small quantity of powder was placed into a fusion tube. That tube was placed in the melting point determining apparatus (Chemline) containing castor oil [47]. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

Table 5: Melting point of Fluconazole

S. No.	Melting Point of Fluconazole
1.	138-140 ℃

Determination of pH (1% w/v solution in water)

Procedure:

About 100mg of the Powder was taken and dissolved in 10ml of distilled water with sonication and filtered. The pH of filtrate was checked with standard glass electrode (Digital pH meter Electronic India).

Table 6: pH of Fluconazole

S. No.	Drug	pH of 1% solution of drug
1.	Fluconazole	7.75

Moisture content determination:

Karl Fischer volumetry is used for samples with high water content, *i.e.* 1-100 mg per sample. An iodine-containing solution serves as titrating agent. The water content of the sample is calculated using titration volume and titer of the titrating agent. Reagents conveniently contain all reactants (iodine, sulfur dioxide and a base) dissolved in a suitable alcohol in one solution, whereas two-component reagents contain all necessary reactants separated in two different solutions to enhance the rapidity of the Karl Fischer reaction and the titer stability of the titrating agent.

Table 7: Moisture content determination

S. No.	Drug	KF Factor	Amount of KF Reagent consumed	Moisture content
1.	Fluconazole	0.523	0.5ml	0.261

Identification test using FTIR Spectroscopy

Infra- red spectrum is an important record which gives sufficient information about the structure of a compound [48]. This technique provides a spectrum containing a large number of absorption band from which a wealth of information can be derived about the structure of an organic compound. The region from 0.8 μ to 2.5 μ is called Near Infra-red and that from 15 μ to 200 μ is called Far infra-red region.

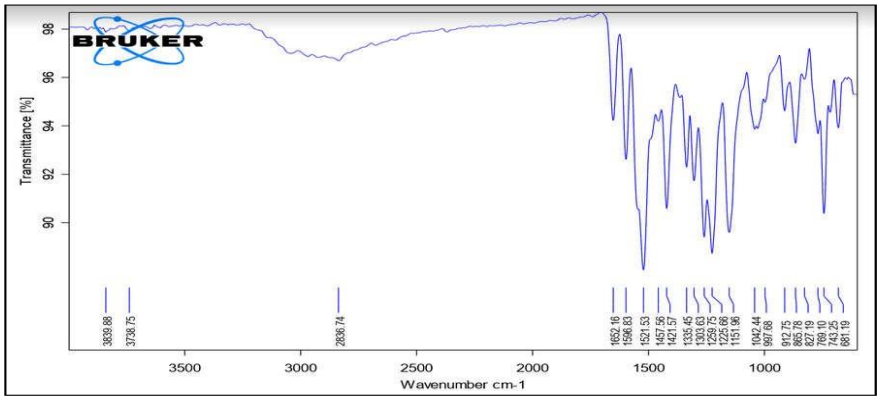

Figure 2: FT-IR Spectrum (Fluconazole)

Determination of λ_{max} of Fluconazole

The λ_{max} of Fluconazole was determined by running the spectrum of drug solution indouble beam ultraviolet spectrophotometer.

Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.2 pH phosphate buffer solutions in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 0.1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.2 pH phosphate buffer solution prepare suitable dilution to make it to a concentration of 10µg/ml for Fluconazole. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+).

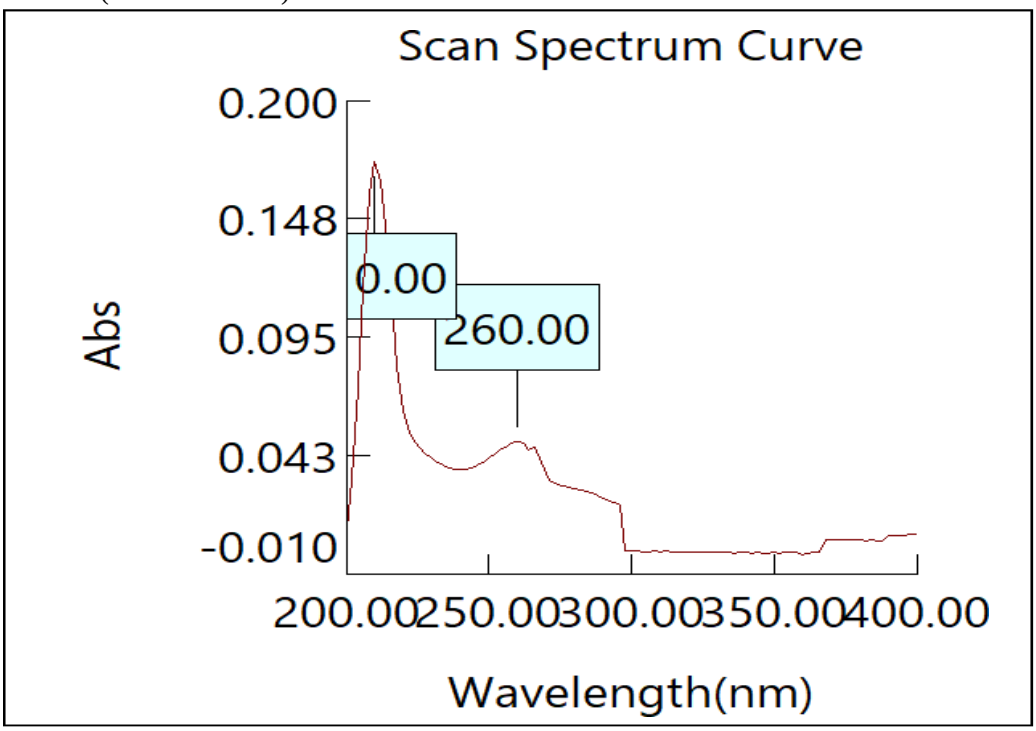

Figure 3: Determination of λ_{max} of Fluconazole

Calibration curve of Fluconazole

Preparation of standard stock solution

10mg of drug was weighed accurately and transferred to 10 ml volumetric flask, and the volume was adjusted to the mark with the 7.2 pH phosphate buffer to give a stock solution of 1000 ppm or µg/ml.

Preparation of working standard solution

From stock solutions of Fluconazole 1 ml was taken and diluted up to 10 ml. from this solution 1.0, 2.0, 3.0, 4.0 and 5.0 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with 7.2 pH phosphate buffer, gives standard drug solution of 10, 20, 30, 40, 50 µg/ml concentration.

Table 8: Readings for Calibration curve of Fluconazole

S. No.	Concentration (µg/ml)	Absorbance
1.	10	0.205
2.	20	0.375
3.	30	0.539
4.	40	0.697
5.	50	0.879

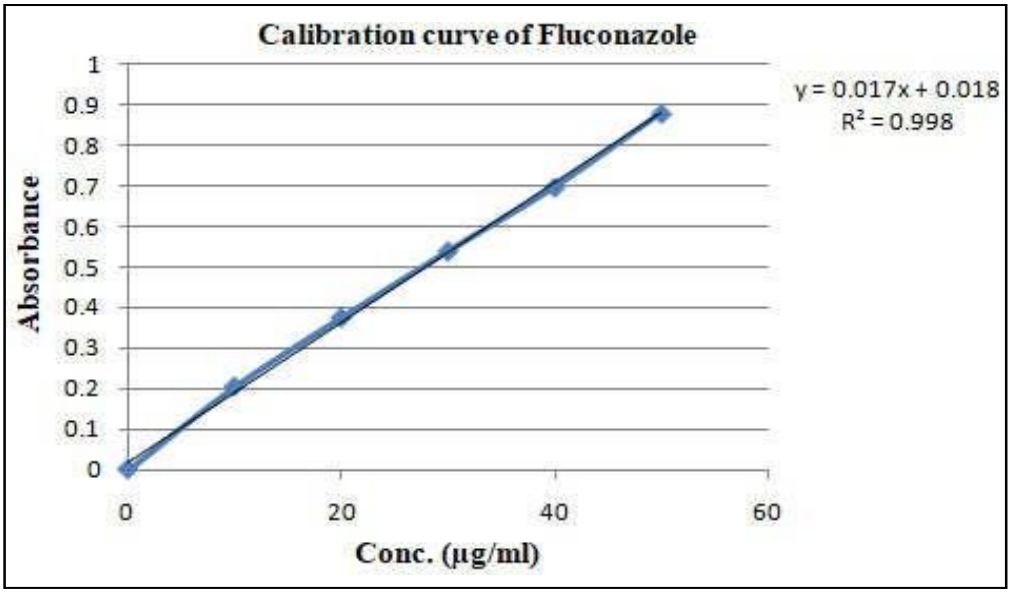

Figure 4: Calibration curve of Fluconazole at 260 nm

Result & Discussion

Table 9: Different Composition of ethosomes formulation

F. Code	Drug (mg)	Phospholipid (% w/v)	Ethanol (% w/v)	PEG (%w/v)	SDS (mg)	Water (%w/v)
F1	500	0.5	10	20	50	100
F2	500	0.5	20	20	-	100
F3	500	1.0	10	20	50	100
F4	500	1.0	20	20	-	100
F5	500	1.5	10	20	50	100
F6	500	1.5	20	20	-	100

Evaluation of Fluconazole loaded Ethosomes

Microscopic observation of prepared ethosomes

An optical microscope (Cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared ethosomes formulation [51].

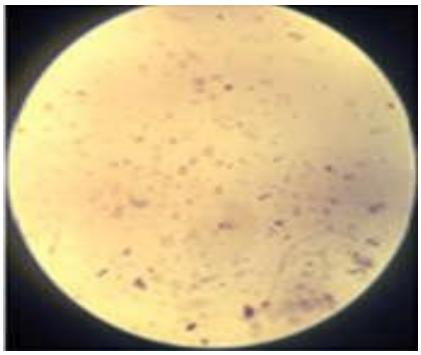

Figure 5: Microscopic observation of prepared ethosomes formulation

Vesicle size and zeta potential

Vesicle size and zeta potential of the Ethosomes were measured by photon correlation spectroscopy using a horiba scientific, nanoparticle analyzer instrumentthe results shown in table 10.

Entrapment efficiency

Table 10: Result for Vesicle size and Entrapment efficiency of drug loaded Ethosomes

Formulation Code	Vesicle size (μ)	% Entrapment Efficiency
F1	210.25±0.25	72.32±0.25
F2	185.65±0.30	71.85±0.32
F3	195.65±0.15	69.98±0.14
F4	148.85±0.14	74.45±0.35
F5	175.65±0.23	69.98±0.32
F6	165.98±0.18	73.32±0.18

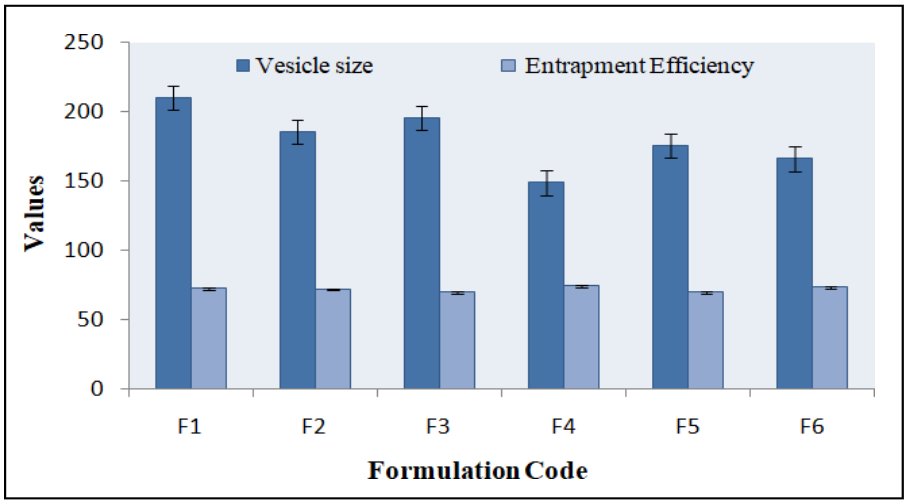

Figure 6: Graphical representation of vesicle size and entrapment efficiency

Table 11: Vesicle size and entrapment efficiency of optimized ethosomes

Formulation Code	Vesicle size (nm)	Entrapment Efficiency	Zeta potential
F4	148.85±0.14	74.45±0.35	-36.45

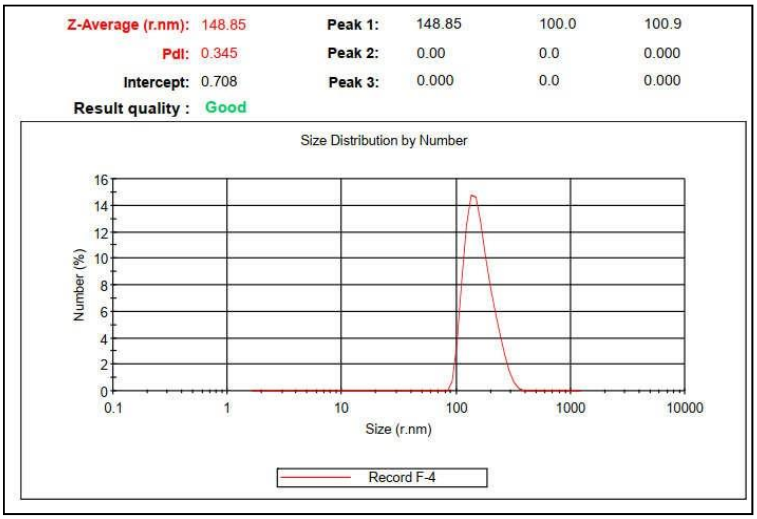

Figure 7: Graph of Vesicle size of optimized formulation F4

Table 12: Composition of different gel base

S. No.	Formulation	Carbopol (%)
1.	EF1	0.5
2.	EF2	1
3.	EF3	2

Evaluation of gel

Table 13: Results of Homogeneity, Extrudability, Spreadability of gel formulation

Code	Homogeneity and Texture	Spreadability (gm.cm/sec.)	Extrudability	Washability
EF1	+++	13.32±0.25	+++	Good
EF2	+++	11.15±0.32	+++	Good
EF3	+++	10.23±0.15	+++	Good

+++ Good ++ Average

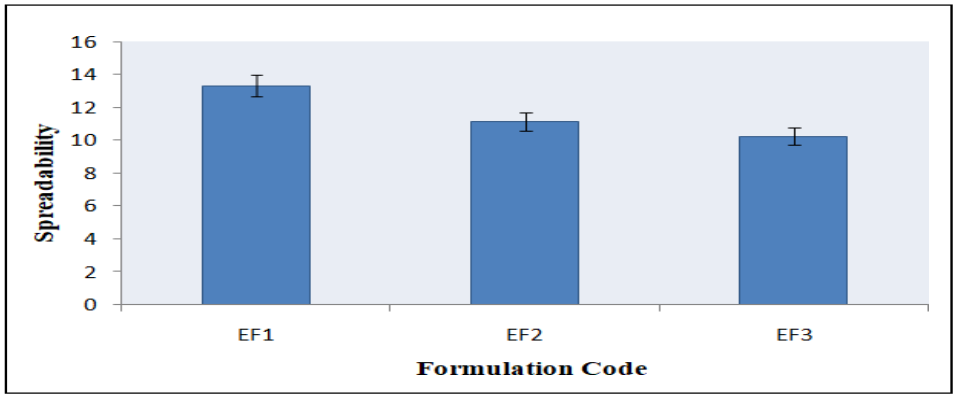

Figure 8: Graph of Spreadability

Table 14: Results of pH, Viscosity and % Assay

Code	pH	Viscosity (cps)	% Assay
EF1	6.74±0.25	3465±15	98.85±0.32
EF2	6.80±0.35	3310±25	99.45±0.25
EF3	6.95±0.36	3025±32	97.74±0.15

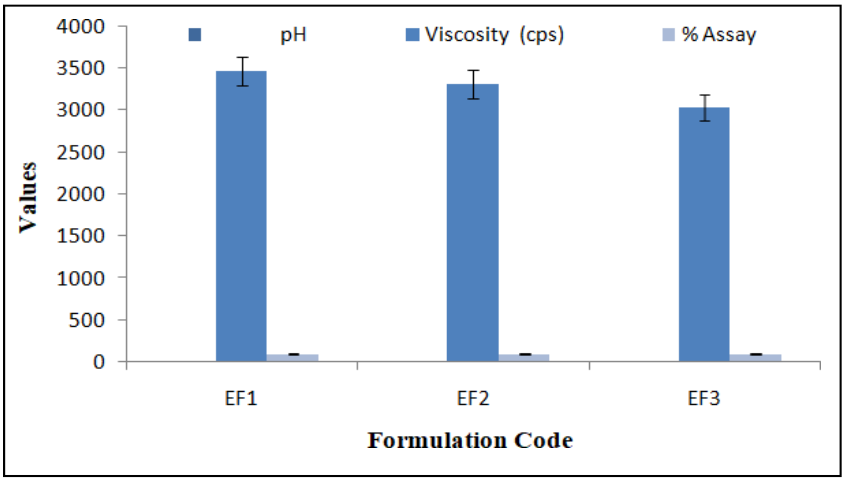

Figure 9: Graph of pH, viscosity and % assay

Table 15: Cumulative % drug release of Fluconazole from optimized ethosomes gel formulation F4

S. No.	Time (hrs)	% Cumulative drug release ethosomal gel
1	0.5	28.65±0.32
2	1	48.98±0.15
3	2	59.98±0.23
4	4	72.23±0.41
5	6	81.15±0.36
6	8	93.35±0.45
7	10	99.05±0.36

Table 16: In Vitro drug release data for the optimized gel formulation EF2

S. No.	Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release ± SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	0.5	0.707	-0.301	28.65	1.457	71.35	1.853
2	1	1	0	48.98	1.690	51.02	1.708
3	2	1.414	0.301	59.98	1.778	40.02	1.602
4	4	2	0.602	72.23	1.859	27.77	1.444
5	6	2.449	0.778	81.15	1.909	18.85	1.275
6	8	2.828	0.903	93.35	1.970	6.65	0.823
7	10	3.162	1	99.05	1.996	0.95	-0.022

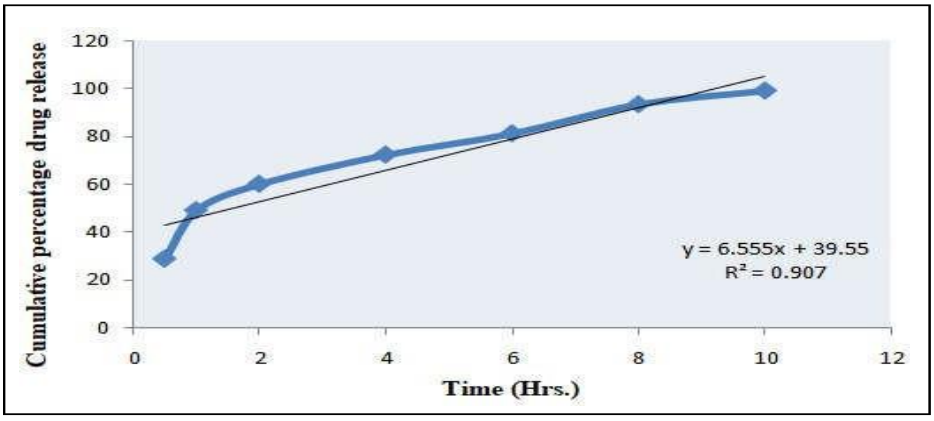

Figure 10: Cumulative percent drug released Vs Time (Zero Order Plots)

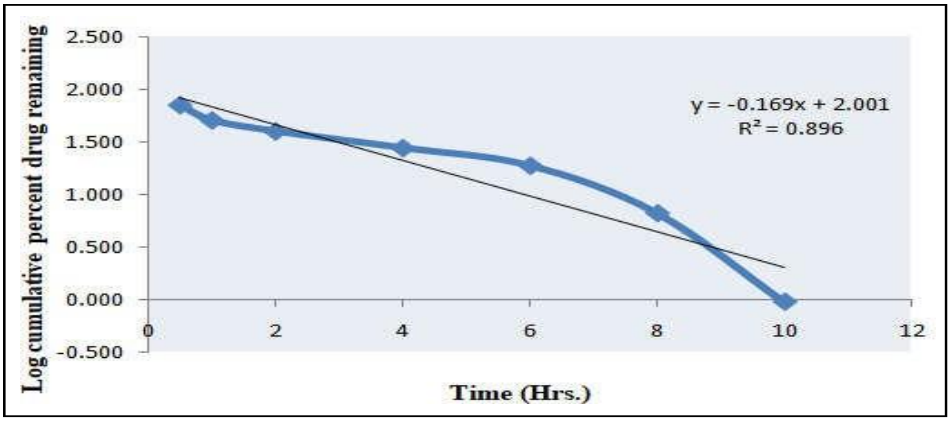

Figure 11: Log cumulative percent drug remaining Vs Time(First Order Plots)

Table 17: Regression Analysis Data of Ethosomal Formulation

Formulation	Zero order	First order	Pappas plot
EF2	$R^2 = 0.907$	$R^2 = 0.896$	$R^2 = 0.959$

Table 18: Characterization of Optimized formulation of gel EF2

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temperature	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C
Percentage drug content	99.00±0.35	98.12±0.15	98.85±0.35	97.75±0.25	98.73±0.32	97.58±0.25
Viscosity	3310±0.21	3295±0.36	3305±0.32	3285±0.14	3300± 0.25	3175±0.36
Physical Appearance	Normal	Highturbid	Normal	High turbid and agglomeration	Normal	High turbid and agglomeration

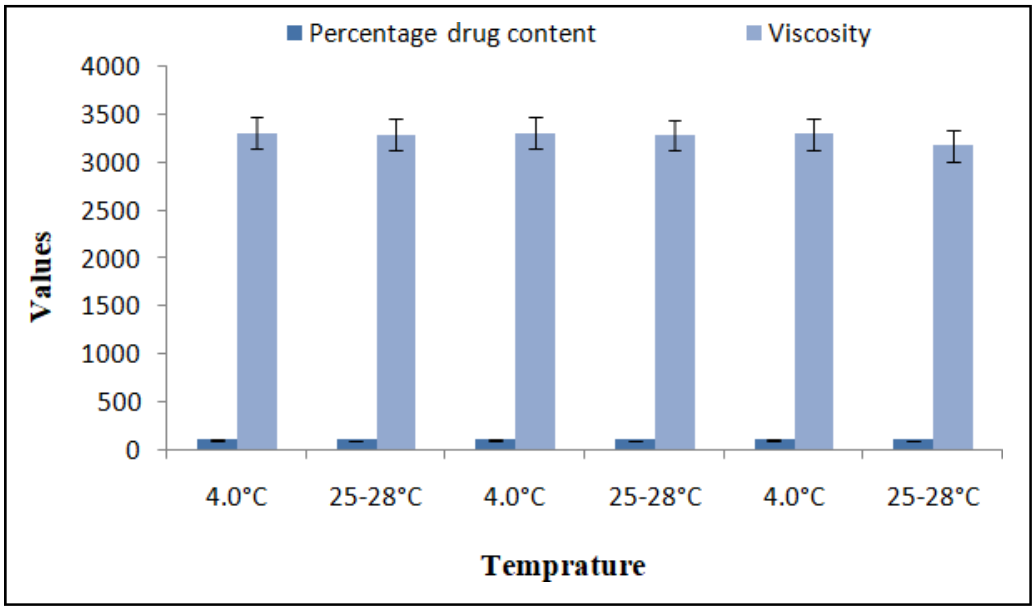

Figure 12: Stability Study

- Preformulation studies are essential protocols for improvement of safety, efficacy and stability of dosage form as well. Thus it plays an important role in order toensure optimum condition for clinically advantageous delivery system.
- Drug substances which irritates to skin should be handle with precautions. Flavors, dyes, excipients used will affect stability and bioavailability of dosage form. The preliminary study showed that Fluconazole is white characteristic powder.
- The melting point of Fluconazole was found to be of 138-140℃. Loss on drying value of Fluconazole was found to be 0.261%.
- Ethosomal formulations were prepared by hand shaking method. Total six formulations were prepared using varying amount of phospholipids and ethanol. With and without SDS. The prepared formulation was evaluated for Microscopic observation, Vesicle size, zeta potential and Entrapment efficiency.
- The maximum entrapment efficiency was observed in formulation F4, was further subjected for zeta potential and particle size. The particle size, Entrapment efficiency and zeta potential was found to be

148.85±0.14, 8674.45±0.35 and - 36.45mv respectively.

- In all formulation formulation EF2 select as a optimized formulation because of its good Spreadability (11.15±0.32), Viscosity (3310±25) and pH (6.80±0.35). The maximum % assay was also found in formulation F2 (99.45±0.25).

Conclusion

Ethosomes offers a good opportunity for the non-invasive delivery of small-, medium-, and large-sized drug molecules. Studies will continue to further improve the skin delivery of drugs using lipid vesicles. Special emphasis seems to be given to the skin delivery of proteins and other macromolecules and for transcutaneous immunization. The near future also holds the emergence of new commercial ethosome-based topical products.

In this study an attempt has been made to formulate a supplement dermal therapy of fluconazole. The ethosomal encapsulation of fluconazole was found to increase the skin residence time leading to a faster healing of external lesions and to a reduction of side effects and duration of therapy. Fluconazole ethosomes prepared by hot technique with required modifications after optimizing formulation variables.

Ethosomes were prepared and optimized on the basis of average vesicle size, and % drug entrapment. The optimized formulation was further encorpoated with gel base (carbapol gel) and characterized for their viscosity, extrudability, spreadability and drug release study. It was found that in all formulation formulation EF2 select as a optimized formulation because of its good Spreadability (11.15±0.32), Viscosity (3310±25) and pH (6.80±0.35). The maximum % assay was also found in formulation EF2 (99.45±0.25). In vitro drug release from ethosomal gel was carried out using franz diffusion cell method and found 99.05% in 10 hr. In first 50 min it was 28.65% drug release which slightly high. It was due to the release of free drug present in bag after leaching from liposomes. Drug release from ethosomes formulation was found in very sustained and controlled manner. It was concluded that prepared gel containing fluconazole loaded ethosomal formulation was optimized and successfully formulated in the form gel can be used for topical preparation for antiacne affect.

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