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Formulation and evaluation of Gel containing Fluconazole-Antifungal Agent

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Abstract

Fluconazole is an imidazole derivative and used for the treatment of local and systemic fungal infection. The oral use of fluconazole is not much recommended as it has many side effects. Commercially fluconazole topical gel preparation are not available in the market, thus this formulation is made for better patient compliance and to reduce the dose of drug and to avoid the side effects like liver damage and kidney damage.. The gel was formulated by changing the polymer ratio. FT-IR study confirmed the purity of drug and revealed no interaction between the drug and excipients. Gel formulations were characterized for drug content, pH determination, viscosity measurement, *in vitro* diffusion, antifungal activity and skin irritation. Among the five formulations, F1 was selected as the best formulation as its %CDR after 4½ h was 97.846% and release rate of drug from F1 formulation is best fitted to Higuchi model. The viscosity of the F1 formulation was within the limits and F1 formulation did not show any skin irritation. Gel formulation F1 was found to be stable at 30 ±2°C and 65 ± 5 RH. It was found that at 40 ± 2°C and 75 ± 5 RH the gel formulation was not stable and %CDR was decreased. Efficient delivery of drug to skin application was found to be highly beneficial in localizing the drug to desired site in the skin and reduced side effects associated with conventional treatment.

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INTRODUCTION

Fungal infection of skin is now-a-days one of the common dermatological problem. The physicians have a wide choice for treatment from solid dosage to semisolid dosage form and to liquid dosage formulation. Among the topical formulation clear transparent gels have widely accepted in both cosmetics and pharmaceuticals[1].

Topical treatment of dermatological disease as well as skin care, a wide variety of vehicle ranging from solids to semisolids and liquids preparations is available to clinicians and patients. Within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparation[2, 3]. Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal, and skin as topical routes. Skin is one of the most accessible organ of human body for topical administration and main route of topical drug delivery system. Number of medicated products is applied to the skin or mucous membrane that either enhances or restores a fundamental function of a skin or pharmacologically alters an action in the underlined tissues. Such products are referred as topical or dermatological products[3, 4]. Hydroxypropyl methylcellulose (HPMC), Carbapol 934p, Sodium alginate has been used as hydrophilic polymers topically in gel drug delivery system[5, 6]. A series of grades based on molecular fractions of these polymers are used at a concentration between 1 to 5% in topical gel formulation. The application of medicinal substance to the skin is a concept doubtless as humanity[5, 6].

It is necessary to understand the anatomy, physiology, physicochemical properties of the skin to utilize the phenomenon of percutaneous absorption successfully. The skin of an average adult human covers a surface area of approximately 2 m^2 and receives about one-third of the blood circulating through the body. Microscopically skin is composed of three main histological layers: Epidermis, Dermis and Hypodermis (subcutaneous layer) [7].

The epidermis is 0.1 – 1.5 mm thick. It is further divided into five parts: stratum germinativum, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum, the epidermis forms the pigment melanin. The squamous cell layer is the thickest layer of the epidermis and helps to move certain substances in and out of the body. The stratum corneum ("horny layers") is made up of 10 to 30 thin layers of the dead cells. The outermost cells are replaced by new layers of cells[7].

The dermis lies just beneath the epidemis, is 1.5 to 4 mm thick. It contains collagen, elastin, sweat and oil glands, hair follicles, nerve endings and blood and lymph vessels. Dermis also acts as storage for water. The dermis also contains scavenger's cells from the immune system which engulf the foreign organism and destroy it. Nerve ending also is found in the dermis which is responsible for the sense of touch[7].

The subcutaneous tissue (hypodermis) is the deepest layer of the skin. Subcutaneous tissue acts as an insulator-conserving body heat, and as a shock absorber protecting internal organs from injury. It also stores fat. The blood vessels, nerves, lymph vessels, and hair follicles also cross through these layers[7,8].

ROUTE OF PENETRATION

At the skin surface, drug molecules come in contact with cellular debris, microorganisms, and other materials, which effect permeation. The applied medicinal substance has three pathways to the viable tissue- 1) through hair follicles, 2) via sweat ducts and 3) across continuous stratum corneum between the appendages (hair follicles, sebaceous glands, eccrine, apocrine glands and nails).

Fractional appendageal area available for transport is only about 0.1% and is important for ions and large polar molecules. The intact stratum corneum is the main barrier and therefore many enhancing techniques aim to disrupt or bypass this layer. Viable layers may metabolize a drug, or activate a prodrug. Usually, deeper dermal regions do not significantly influence absorption.

For more than two decades, researchers have attempted to find a way to use the skin as a portal of entry for drugs in order to overcome problems associated with traditional mode of drugs

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administration. This route of drug delivery has gained popularity because it avoids first-pass effect, gastrointestinal irritation and metabolic degradation associated with oral administration. The topical route of administration has been utilized either to produce local effect for treating skin disorder or to produce systemic drug effects[9, 10].

In treating skin disease, the primary purpose of applying drug to the skin is to induce local effect at the site of application. In most of the cases, only a small portion of dose finally reaches the site of action, and produce limited local activity. This has been a complicated task due to the highly effective barrier properties of the skin.

The fungal infections are very common and can be topical as well as systemic. The fungal infections can be treated by topically applied medicines as well as by oral administrations. However, oral use of medicine is not much important in treating local fungal infections and also has systemic side effects.

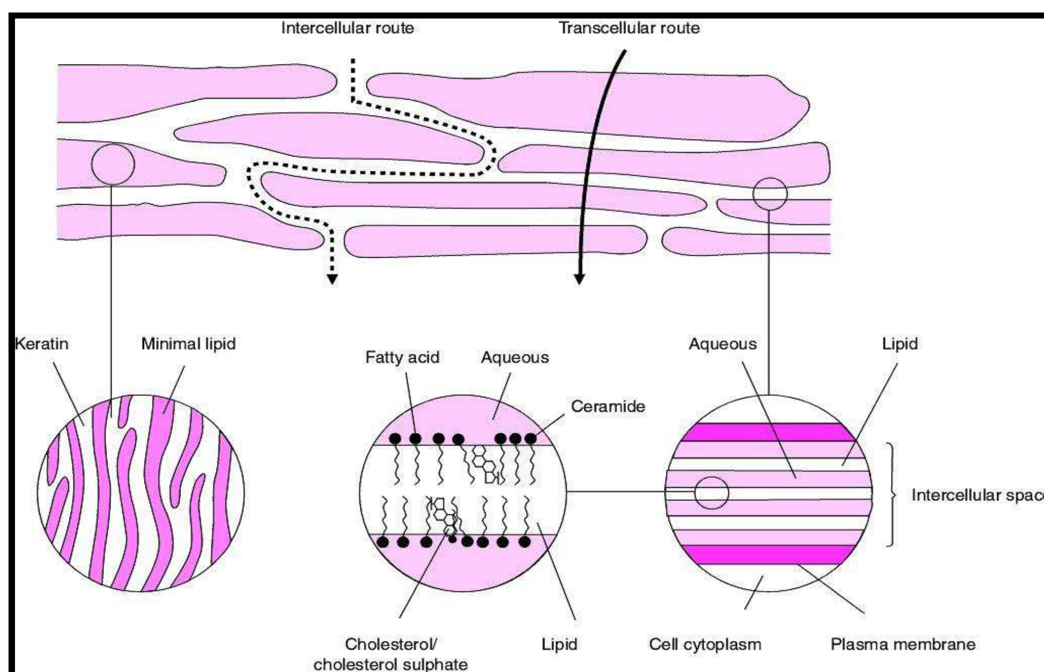

Figure 1: Simplified diagram of stratum corneum and two micro routes of drug penetration

Treatment of fungal infections includes following medicines: Fluconazole, Ketoconazole, Clotrimazole, Itraconazole, Miconazole, and Griseofulvin. Fluconazole is a synthetic antifungal agent of the imidazole class; it works by slowing the growth of fungi that cause infection. It is used to treat fungal infection.

Gels are defined as "semisolid system in which a liquid phase is constrained within a polymeric matrix in which a high degree of physical and chemical cross-linking introduced".

CLASSIFICATION OF GELS

Gels can be classified based on colloidal phases, nature of solvent used, physical nature and rheological properties.

1. BASED ON COLLOIDAL PHASES:

They are classified into

- Inorganic (two phase system)
- Organic (single phase system)

Two phase system:

If partial size of the dispersed phase is relatively large and form the three-dimensional structure throughout gel, such a system consists of floccules of small particles rather than larger molecules and gel

structure, in this system is not always stable. They must be thixotropic-forming semisolids on standing and become liquid on agitation.

Single-phase system:

These consist of large organic molecules existing on the twisted strands dissolved in a continuous phase. This larger organic molecule either natural or synthetic polymers are referred as gel formers, they tend to entangle with each other their random motion or bound together by Vander walls forces.

2. Based on nature of solvent:

Hydro gels (water based):

Here they contain water as their continuous liquid phase

E.g.: bentonite magma, Gelatin, cellulose derivatives, carpooler, and poloxamer gel.

Organic Gels (with a non-aqueous solvent):

These contain a non-aqueous solvent on their continuous phase.

E.g. plastibase (low molecular wt polyethylene dissolved in mineral oil & short Cooled) Olag (aerosol) gel and dispersion of metallic stearate in oils

Xerogels:

Solid gels with low solvent concentration are known as xerogels. These are produced by evaporation of solvent or freeze drying, leaving the gel framework behind on contact with fresh fluid, they swells and can be reconstituted.

E.g. Tragacanth ribbons, acacia tear β-cyclodextrin, dry cellulose and polystyrene.

3. Based on rheological properties:

Usually gels exhibit non-Newtonian flow properties. They are classified into,

a) Plastic gels

b) Pseudo plastic gels

c) Thixotropic gels.

(a) Plastic gels:

E.g. - Bingham bodies, flocculated suspensions of Aluminum hydroxide exhibit a plastic flow and the plot of rheogram gives the yield value of the gels above which the elastic gel distorts and begins to flow.

(b) Pseudo-plastic gels:

E.g.: - Liquid dispersion of tragacanth, sodium alginate, Na CMC etc. exhibits pseudo-plastic flow. The viscosity of these gels decreases with increasing rate of shear, with no yield value. The rheogram results from a shearing action on the long chain molecules of the linear polymers. As the shearing stress is increased the disarranged molecules begin to align their long axis in the direction of flow with release of solvent from gel matrix.

(c) Thixotropic gels:

The bonds between particles in these gels are very weak and can be broken down by shaking. The resultant solution will revert back to gel due to the particles colliding and linking together again. (The reversible isothermal gel-sol-gel transformation). This occurs in colloidal system with non-spherical particles to build up a scaffold like structure.

E.g.: Kaolin, bentonite and agar.

4. Based on physical nature:

(a) Elastic gels:

Gels of agar, pectin, Guar gum and alginates exhibit an elastic behaviour. The fibrous molecules being linked at the point of junction by relatively weak bonds such as hydrogen bonds and dipole attraction. If the molecule possesses free –COOH group then additional bonding takes place by salt bridge of type –COO-X-COO between two adjacent strand networks.

E.g.: Alginate and Carbapol.

(b) Rigid gels:

This can be formed from macromolecule in which the framework linked by primary valance bond.

E.g.: In silica gel, silic acid molecules are held by Si-O-Si-O bond to give a polymer structure possessing a network of pores.

PREPARATION OF GELS:

Gels are normally in the industrial scale prepared under room temperature. However few of polymers need special treatment before processing. Gels can be prepared by following methods.

1. Thermal changes
2. Flocculation
3. Chemical reaction

1) Thermal changes:

Solvated polymers (lipophilic colloids) when subjected to thermal changes causes gelatin. Many hydrogen formers are more soluble in hot than cold water. If the temperature is reducing, the degree of hydration is reduced and gelatin occurs. (Cooling of a concentrated hot solution will produce a gel).

E.g.: - Gelatin, agar sodium oleate, guar-gummed and cellulose derivatives etc. In contrast to this, some materials like cellulose ether have their water solubility to hydrogen bonding with the water. Raising the temperature of these solutions will disrupt the hydrogen bonding and reduced solubility, which will cause gelation. Hence this method cannot be adopted to prepare gels as a general method.

2) Flocculation:

Here gelation is produced by adding just sufficient quantity of salt to precipitate to produce age state but insufficient to bring about complete precipitation. It is necessary to ensure rapid mixing to avoid local high concentration of precipitant.

E.g.: Solution of ethyl cellulose, polystyrene in benzene can be gelled by rapid mixing with suitable amounts of a non-solvent such as petroleum ether. The addition of salts to hydrophobic solution brings about coagulation and gelation is rarely observed. The gels formed by flocculation method are Thixotropic in behaviour. Hydrophilic colloids such as gelatin, proteins and acacia are only affected by high concentration of electrolytes, when the effect is to "salt out", the colloidal and gelation doesn't occur.

3) Chemical reaction:

In this method gel is produced by chemical inter action between the solute and solvent.

E.g.: aluminium hydroxide gel can be prepared by interaction in aqueous solution of an aluminium salt and sodium carbonate an increased concentration of reactants will produce a gel structure. Few other examples that involve chemical reaction between PVA, cyanoacrylates with glycidol ether (Glycidol), toluene diisocyanates (TDI), methane diphenyl isocyanine (MDI) that cross-links the polymeric chain

TYPES OF FUNGAL DISEASE

- Skin infection: e.g. foot fungus (usually smelly but not life threatening, sometimes becomes serious), ring worms.
- Mucosal infections: oral or vaginal (range from annoying to painful to very difficult; uncomfortable but rarely life threatening).
- Systemic infection: fungus in the blood and tissues (immunocompromised population, usually life threatening) [11].

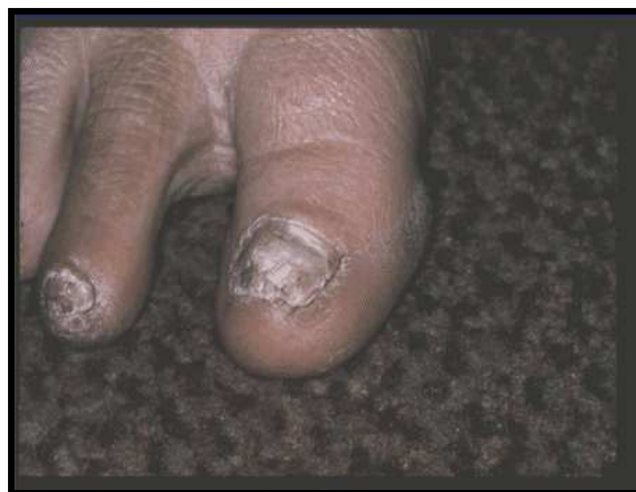

Figure 2: Onychomycosis: foot fungus

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Different classes of drugs target the plasma membrane, sterol biosynthesis, DNA biosynthesis, and β-glucan biosynthesis. Fungal membranes and sterol biosynthetic enzymes are different enough from ours that these agents can kill fungi but not us. Fungi make β-glucan, we don't, so drugs that target β-glucan biosynthesis have low side-effects[11].

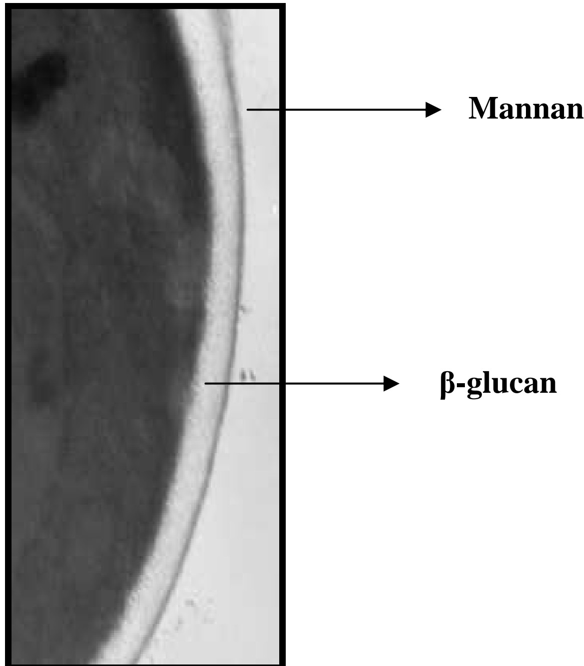


Figure 3: Fungal membrane showing Mannan and β-glucan

Two main fungal-specific molecules are β-glucan and mannan, chains of sugars linked in particular order. Immune receptors bind to these molecules and begin a choreographed immune response. A productive immune response is tiered: first immune cells signal an invasion and recruit more immune cells to the site of infection, and then these cells kill the fungus and stimulate a long-lived response that protects against future infection[11].

Recognition of β-glucan stimulates the anti-fungal immune response which causes phagocytosis of the fungus (leads to killing). Production of attractive and activating signalling molecules. Priming of the adaptive (memory) arm of the immune system to develop fungal-specific antibodies and T-cells[11].

MECHANISM OF ACTION

- Triazole drug targets the fungal-specific synthesis of membrane lipids.
- Fluconazole inserts preferentially into fungal membranes and disrupts their function.

5-fluorocytosine targets fungal specific DNA replication[11].

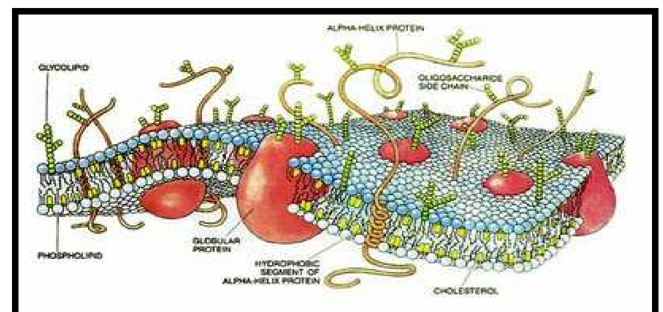
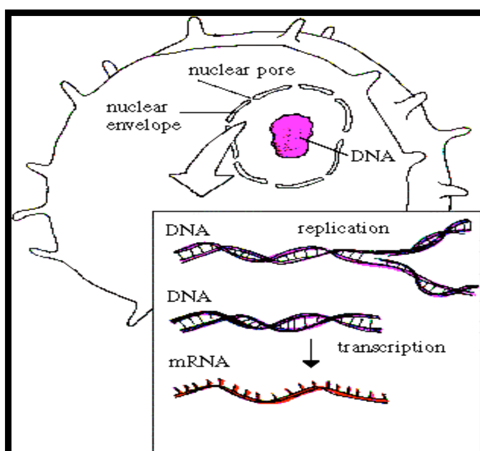


Figure 4: Fungal membrane



5-fluorocytosine targets fungal specific DNA replication

Figure 5: 5-fluorocytosine targets fungal specific DNA replication

INTRODUCTION OF DRUG

Fluconazole is an antifungal drug; fluconazole fights opportunistic infections in people with HIV, severe fungal infection.

- Fluconazole is antifungal agent of triazole class.
- It is new existing drug.
- It overcomes all the side effects of the other fungal drugs like, Ketoconazole, Amphotericin B, Clotrimazole, and Miconazole.
- Even though it has some of the side-effects in the oral and I.V dosage forms.

Fluconazole remains one of the most frequent prescribed triazoles because of its excellent bioavailability, tolerability, and side-effect profile. More than 80 % of ingested drug is found in the circulation, and 60 to 70% is excreted in the urine. Only 10% of fluconazole is protein bound[12].

Fluconazole also exhibits excellent tissue penetration. CSF levels are 70% of matched serum levels, and levels reported in saliva, sputum, and other sites are well within therapeutic ranges. The half-life is 27 to 34 h in the presence of normal renal function allowing once-daily dosing. In patients who have a reduced creatinine clearance the normal dose should be reduced by 50%. Fluconazole serum levels are rarely necessary. Currently 50, 100, 150, and 200 mg tablets are available and IV formulation exits in 200 or 400 mg doses[13, 14].

Available dosage forms:

- Tablets
- Capsule
    - But the gel dosage of this antifungal agent was not formulated.
    - Numerous dosage forms are used in the topical treatment of superficial fungal infections, including creams, liquids, gels, ointments, lacquers and others. The treatment of athlete's foot and ringworm can easily be accomplished with creams, liquids, gels and ointments.

Side effects:

When fluconazole overcomes side effects of other antifungal agents, it also has some side effects in the oral and parentrals dosage forms as pass through the 1st pass metabolism through the liver and excretion through kidneys.

- Headache
- Diarrhea
- Nausea
- Dizziness
- Stomach pain
- Change in the way food tastes.
- Liver and Kidney damage.
- The most common side effects of fluconazole are headache, nausea and pain in the abdomen.
- A few people get diarrhoea, most anti-HIV medications cause problems in the digestive system. Fluconazole could make those problems worse.
- Fluconazole can be hard on the liver.
- Fluconazole can also cause kidney damage.

Due to these side effects of tablet dosage of fluconazole drug the gel dosage form was formulated which was not yet marketed in India.

METERIALS AND METHODOLOGY

Materials:

Materials	Source
Fluconazole	Bal Pharma Pvt.Ltd, Banglore.
Carbopol 934p	Loba Chemie Pvt.Ltd, Mumbai.
Ethanol	Karnataka fine chem, Banglore.
Methyl paraben	Rolex Chemical industries, Mumbai
Propyl paraben	NR Chemicals industries, Mumbai
Propylene glycol	Karnataka fine chem, Banglore

Table 2: List of materials

Equipment	Model / Company
Electron analytical balance	Electron balance, Shimadzu, Japan.
UV-visible spectrophotometer	Spectrophotometer UV-1700, Shimadju.
Fourier Transform Infrared Spectroscope	Tensor 27, Bruker optics.
Magnetic stirrer	2-ML Remi equipment Pvt.Ltd
pH Tutor	EUTECH instrument
Franz diffusion cell	Sci. Work, Peenya 1st stage, Bengaluru.
Ultra sonicator	Sonics and materials inc, USA
Brookfield viscometer	PRO-II extra model, Brookfield viscometer, USA

Table 3: List of equipments

Drug-Excipients Compatibility Studies: [36]

Drug-excipients compatibility studies were carried out using FT-IR infrared spectrum of pure drug was seen in between 600 to 3800 cm^{-1}. The study was carried out on individual pure drug and its physical mixture with the excipients used in the study.

UV Spectrum analysis of Fluconazole:

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

PREPARATION OF STANDARD GRAPH: [36]

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 µg/ml.

Standard Graph of Fluconazole:

Form this standard stock solution, a series of dilution (10, 20, 30, 40, 50 µg/ml) were prepared using methanol. The absorbance of these solutions was measured spectrophotometrically against blank of methanol at 260 nm for fluconazole.

PREPARATION OF GEL BASE: [37]

Carbopol 934p (1, 2, 3, 4, 5% w/w) and purified water were taken in a beaker and allowed to soak for 24 h. To this required amount of drug (2 gm) was dispersed in water and then Carbopol 934p was then neutralized with sufficient quantity of Triethanolamine. Glycerine as moistening agent, methyl paraben and Propyl paraben as preservatives were added slowly with contineous gently stirring untill the homogenous gel was formed.

EVALUATION OF FLUCONAZOLE GEL:

Percentage yield:

The empty container was Weighed in which the gel formulation was stored then again the container was weighed with gel formulation. Then substracted 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.

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

Drug content: [38]

Weighed 10 gm of each gel formulation were transfered in 250 ml of volumetric flask containing 20 ml of alcohol and stirred for 30 min. The volume was made up to 100 ml and filtered. 1 ml of above solution was further diluted to 10 ml with alcohol and again 1 ml of the above solution was further diluted to 10 ml with alcohol. The absorbance of the solution was measured spectrophotometrically at 260 nm.

Drug content was calculated by the following formula.

$$\textbf{Drug content} = \frac{\textbf{Absorbance}}{\textbf{Slope}} \times \textbf{Dilution factor} \times \frac{1}{1000}$$

Dermination of pH:

Weighed 50 gm of each gel formulation were transfered in 10 ml of beaker and measured it by using the digital pH meter.

pH of the topical gel formulation should be between 3 – 9 to treat the skin infections.

Spreadability: (39)

The spreadability of the gel formulation was determined, by measuring diameter of 1 gm gel between horizontal plates (20×20 cm^2) after 1 minute. The standardized weight tied on the upper plate was 125 gm.

Viscosity Estimation: (40)

The viscosity of gel was determined by using a Brookfield viscometer DVII model with a T-Bar spindle in combination with a helipath stand.

a) Selection of spindle:

Spindle T 95 was used for the measurement of viscosity of all the gels.

b) Sample container size:

The viscosity was measured using 50 gm of gel filled in a 100ml beaker.

c) Spindle immersion:

The T-bar spindle (T95) was lowered perpendicular in the centre taking care that spindle does not touch bottom of the jar.

d) Measurement of viscosity:

The T-bar spindle (T95) was used for determining the viscosity of the gels. The factors like temperature, pressure and sample size etc. Which affect the viscosity was maintained during the process. The helipath T- bar spindle was moved up and down giving viscosities at number of points along the path. The torque reading was always greater than 10%. The average of three readings taken in one minute was noted as the viscosity of gels.

***In vitro* diffusion study:** (41)

The abdominal skin of Albino mice, weighing 20 – 25 gm of 8 – 10 week old was shaved using hand razor and clean the skin with hot water cotton swab. 5 gm of gel was applied uniformly to skin. The skin was mounted between the compartments of the Frantz diffusion cell with stratum corneum facing the donor compartment. Reservoir compartment was filled with 100 ml phosphate buffer of pH 6.8.

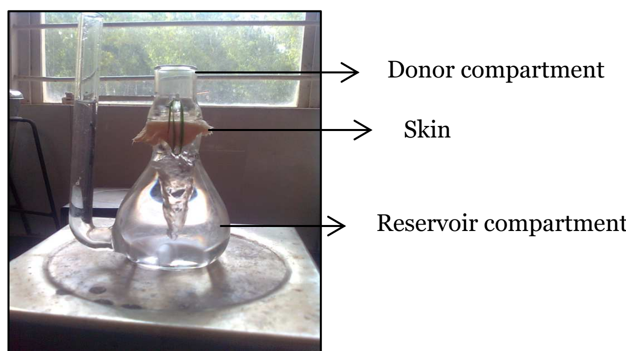


Figure 6: Franz diffusion cell with skin mounted between compartments

The study was carried out at 37 ± 1°C and speed was adjusted untill the vortex touches the skin and it carried out for 4½ h. 5 ml of sample was withdrawn from reservoir compartment at 30 min interval and absorbance was measured spectrophotometrically at 260 nm. Each time the reservoir compartment was replenished with the 5 ml volume of phosphate buffer pH 6.8 solution to maintain constant volume.

Skin irritation study:

This study was carried out on healthy wistar rats. The animals were divided into two group's i.e. control, Gel formulations F1. The back skin of area 5 cm^2 was shaved before one day of starting the study. The study was carried out for 4 days. At the end of study, the animals were observed for any skin irritation like erythema or edema and score were given as per the irritation.

Score	Description
0	No irritation.
0.5	Faint, barely perceptible erythema or slight dryness.
1	Faint but definite erythema, no eruption or broken skin or no erythema but, definite dryness and may have epidermal fissuring.
1.5	Well defined erythema or faint erythema with definite dryness, may have epidermal fissuring.
2	Moderate erythema: may have few papules or erythema in the cracks.
2.5	Moderate erythema with barely perceptible edema.
3	Severe erythema (beet redness) may have generalized papules or moderate to severe erythema with slight edema (edges well defines by raising).
3.5	Moderate to severe erythema with moderate edema (confined to patch area).
4	Generalized vesicles or Escher formation or moderate to severe erythema and/or edema extending beyond the patched area.

Table 4: Scores for skin irritation

- If the formulation produces score of 2 or less, then it is considered to have no irritation.

Anti-fungal studies: (42)

Weighed 16.25 gm of sabouraud dextrose agar was transfered in a 500 ml of conical flask and 250 ml of purified water and some amount of heat is applied to dissolve it completely. Sterilized for 15 min at 121°C at 15 lb pressure in autoclave for about 20 min. Then cooled it at room temperature and the fungal strain(*Candida albicans*) was dispersed in the medium and then the medium was poured it in to the three petridish and allowed it cool it for sometime at room temperature untill it forms solidifies at room temperature and then the three cups are bored in each petridish with the help of sterile steel bore of 6 mm and calculated concentration of the standard drug (Fluconazole), gel formulation(F1) and placebo gel were placed in the bores and incubated the petri plates for 72 h at 37°C in incubators. Then the zone of inhibition was observed and calculated the radius of the zone of inhibition.

Stability studies: (42, 43)

Stability testing of drug product being as a part of drug discovery and ends with the commercial product, to assess the drug and formulation stability, stability studies were done. The stability study was carried out for the most satisfactory formulation. The most satisfactory formulation was sealed in a glass vial and kept at 30 ± 2°C and 40 ± 2°C at RH 65 ± 5 and 75 ± 5 RH for 2 months. At the end of 1 and 2 months, the samples were analyzed for the drug content and *in vitro* diffusion study.

RESULTS

STANDARD GRAPH OF FLUCONAZOLE

Sr. No.	Concentration (µg / ml)	Absorbance at 260 nm ± S.D
1	0	0
2	10	0.262 ± 0.012
3	20	0.448 ± 0.006
4	30	0.633 ± 0.014
5	40	0.823 ± 0.016
6	50	0.999 ± 0.017

Table 5: Standard graph of fluconazole

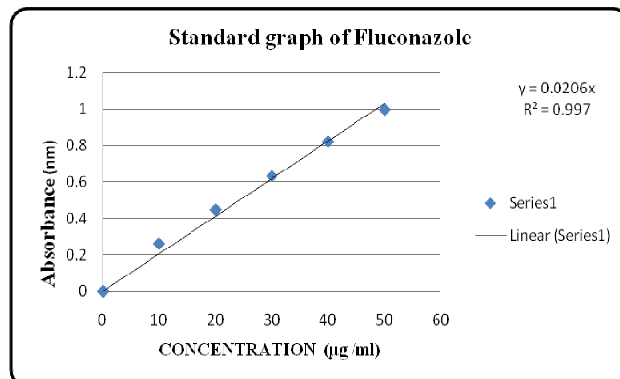


Figure 7: Standard graph of Fluconazole

DRUG-EXCIPIENTS COMPATIBILITY STUDIES BY FT-IR:

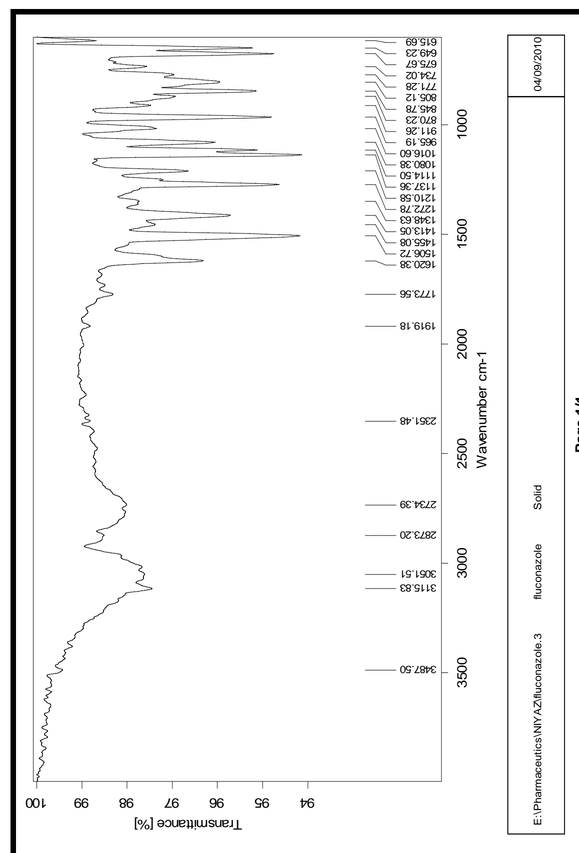


Figure 8: FT-IR Spectra of Fluconazole

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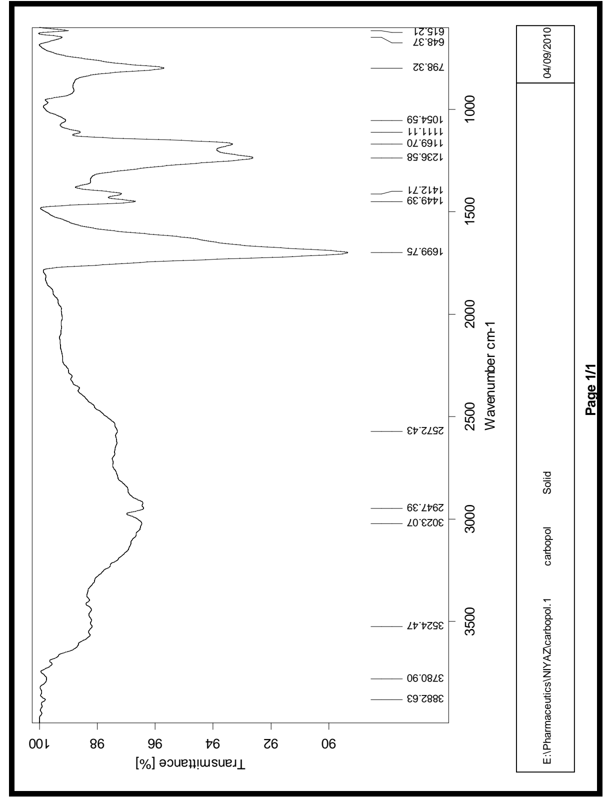

Figure 9: FT-IR Spectra of Carbapol 934p

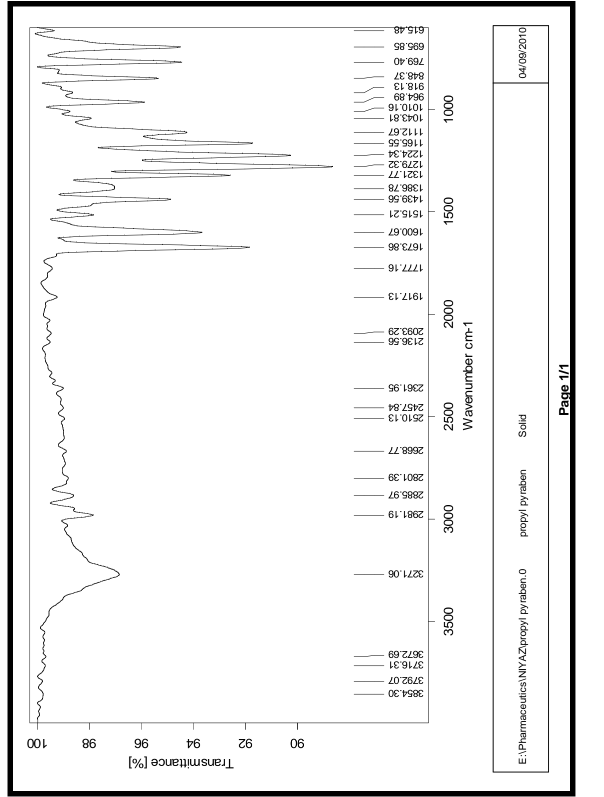

Figure 11: FT-IR Spectra of Propyl paraben

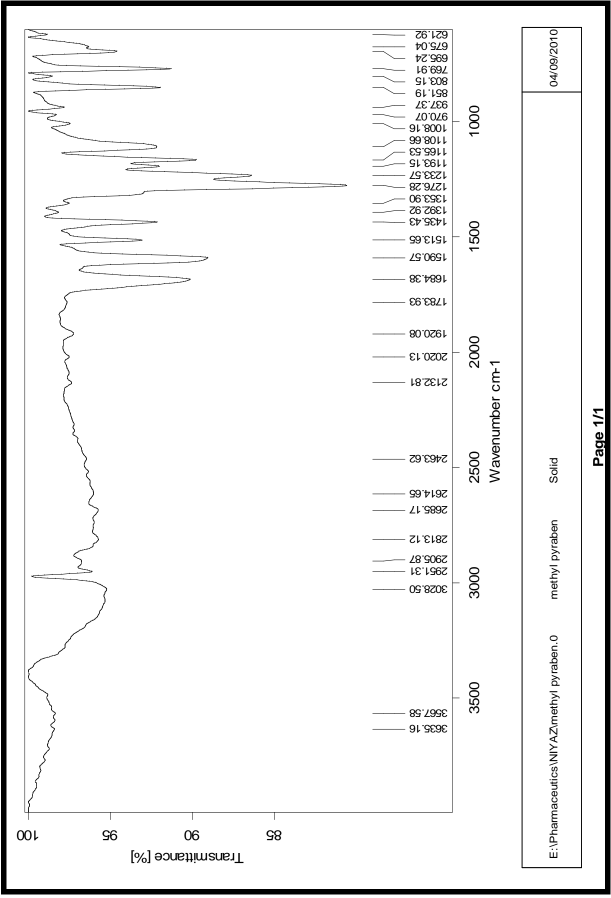

Figure 10: FT-IR Spectra of Methyl paraben

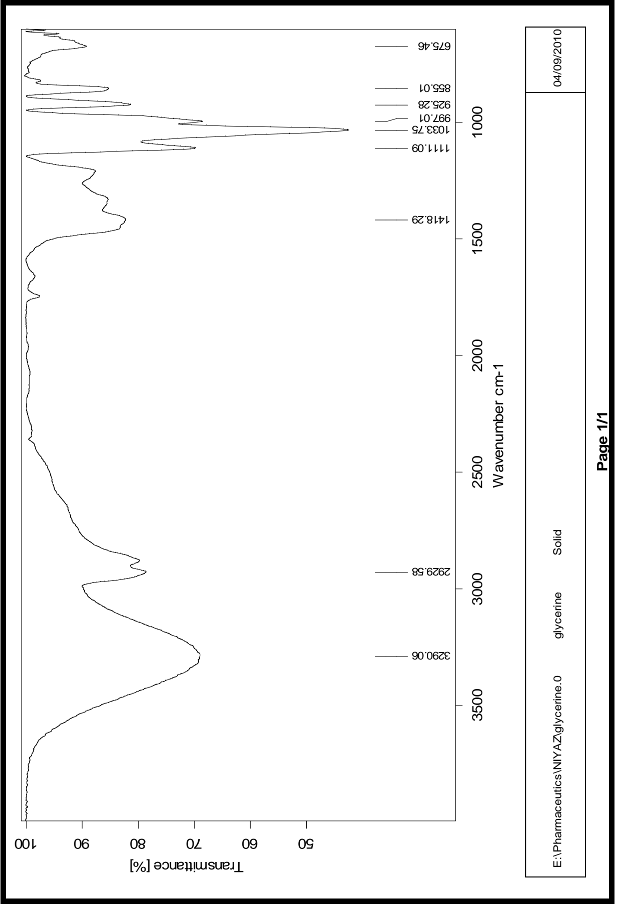

Figure 12: FT-IR Spectra of Glycerine

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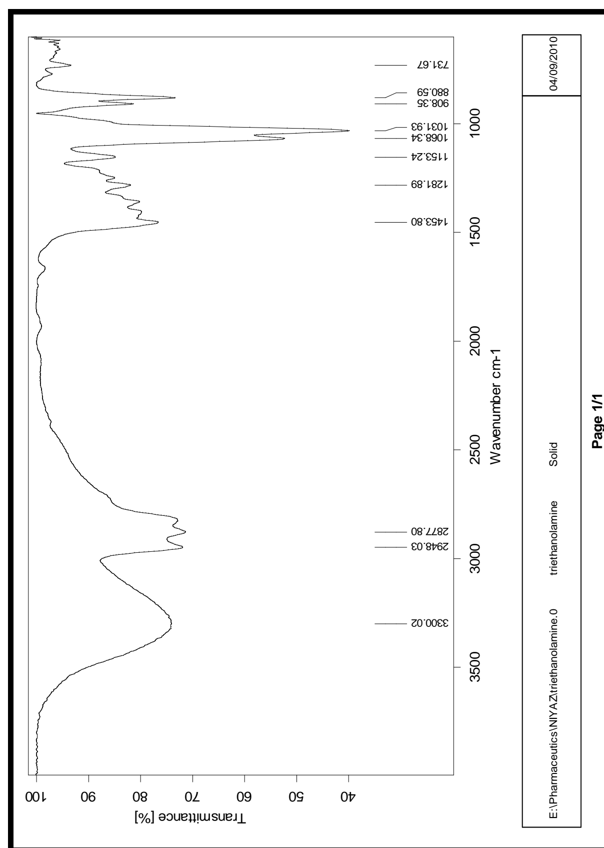

Figure 14: FT-IR Spectra of Triethanolamine

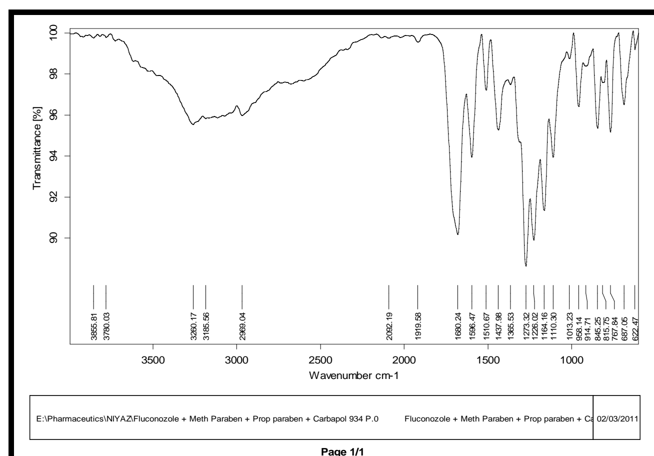

Figure 14: FT-IR Spectra of Fluconazole + Carbapol 934p + propyl paraben + methyl paraben

Peak obtained in drug (frequency cm^{-1})	Description	Peak obtained in mixture (frequency cm^{-1})
3487.50	OH Stretching	3780.03
2873.20	CH2 Stretching	2969.01
3051.51	CH (Aromatic Stretching)	3185.56
1620	C = N Stretch	1596.47
1456	CH (Aromatic bending)	1437.98
870.23	C - F Stretch	914.71

Table 6: Comparison between peaks obtained in drug and in a mixture

FORMULATION CHART:

INGREDIENTS	F1	F2	F3	F4	F5
Drug (gm)	2	2	2	2	2
Alcohol (ml)	4	4	4	4	4
Carbopol 934p (gm)	1	2	3	4	5
Water (ml)	60	60	60	60	60
Methyl paraben (gm)	0.1	0.1	0.1	0.1	0.1
Propyl paraben (gm)	0.05	0.05	0.05	0.05	0.05
Glycerine (ml)	10	10	10	10	10
Triethanol amine (ml)	4	4	4	4	4

Table 7: Formulation chart of gel formulations

PERCENTAGE YIELD:

FORMULATON	PERCENTAGE YIELD %
F1	96.992
F2	98.876
F3	98
F4	96.856
F5	97

Table 8: Percentage yield of gel formulations

DRUG CONTENT:

FORMULATON	DRUG CONTENT %
F1	97 ± 0.027
F2	98 ± 0.027
F3	97.5 ± 0.017
F4	98 ± 0.012
F5	97 ± 0.018

Table 9: Drug content of gel formulations

pH DETERMINATION:

FORMULATON	pH
F1	6.8
F2	7.1
F3	6.9
F4	6.8
F5	7.0

Table 10: pH determination of gel formulations

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SPREADABILITY TEST:

FORMULATON	SPREADABILITY gm.cm²
F1	11.75
F2	11.08
F3	10.75
F4	10.70
F5	10.25

Table 11: Spreadability test of gel formulations

MEASUREMENT OF VISCOSITY:

FORMULATON	VISCOSITY (cps)
F1	65,240.06
F2	92,467.03
F3	97462.37
F4	1,24,000.01
F5	1,40,647.30

Table 12: Measurement of viscosity of gel formulations

IN VITRO DIFFUSION CHART:

TIME (Min)	%CDR				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
30	13.65 ± 0.015	16.422 ± 0.763	14.668 ± 0.712	13.429 ± 0.669	10.353 ± 1.441
60	38.964 ± 1.249	32.072 ± 0.489	30.697 ± 0.834	30.711 ± 0.445	21.283 ± 0.256
90	45.892 ± 2.205	40.544 ± 2.322	40.588 ± 1.232	40.375 ± 0.473	35.755 ± 0.236
120	55.719 ± 1.103	55.324 ± 1.018	56.437 ± 1.240	52.048 ± 0.714	53.137 ± 1.121
150	63.7 ± 0.221	61.701 ± 1.705	62.107 ± 0.313	59.402 ± 0.282	63.688 ± 0.859
180	72.533 ± 1.269	70.859 ± 0.706	69.934 ± 0.386	66.21 ± 0.190	71.121 ± 0.634
210	79.610 ± 0.939	77.923 ± 1.411	74.815 ± 0.493	71.719 ± 0.200	74.502 ± 0.035
240	89.318 ± 0.900	86.266 ± 0.339	84.127 ± 0.079	81.739 ± 0.782	80.149 ± 1.252
270	97.846 ± 0.966	94.741 ± 0.703	93.11 ± 1.145	88.96 ± 0.671	89.439 ± 0.994

Table 13: *In vitro* diffusion studies of gel formulations

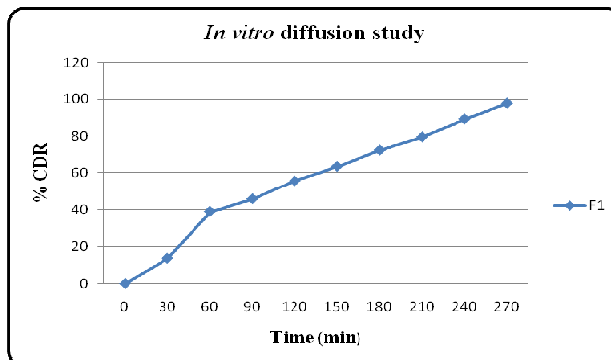

Figure 15: *In vitro* diffusion study of F1

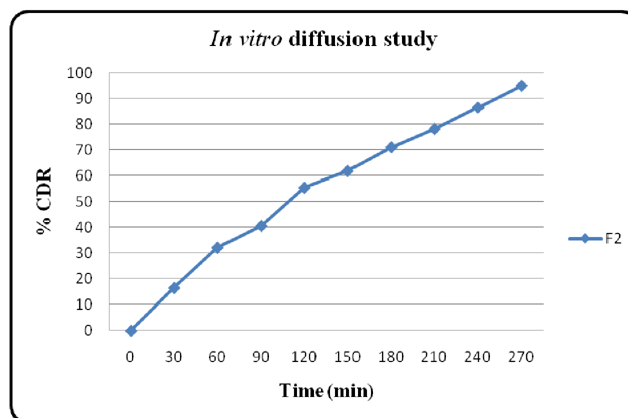

Figure 16: *In vitro* diffusion study of F2

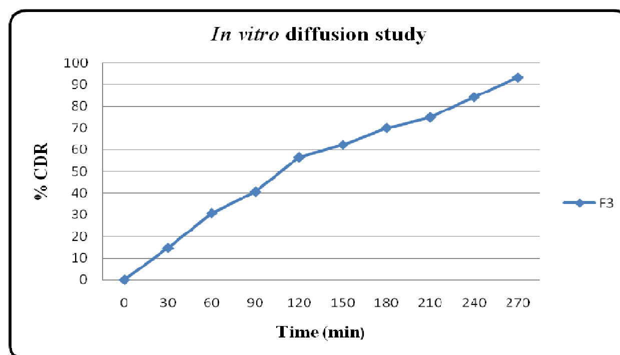

Figure 17: *In vitro* diffusion study of F3

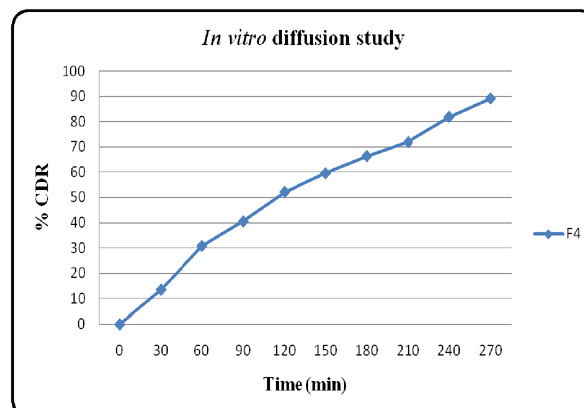

Figure 18: *In vitro* diffusion study of F4

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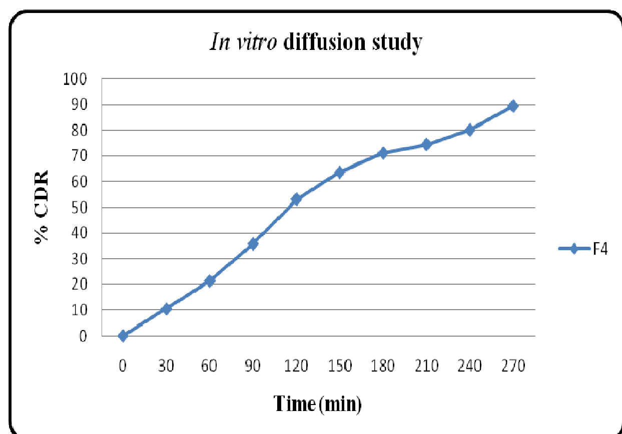

Figure 19: *In vitro* diffusion study of F5

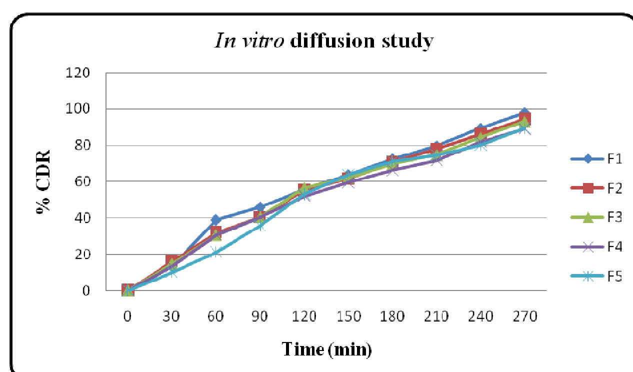

Figure 20: *In vitro* diffusion study of F1, F2, F3, F4, and F5

Drug release kinetics:

Formulations code	r^2			
	Zero order kinetics	First order kinetics	Higuchi model	Korsemeyer-Peppas model
F1	0.970034	0.825179	0.989549	0.953489

Table 14: drug release kinetics

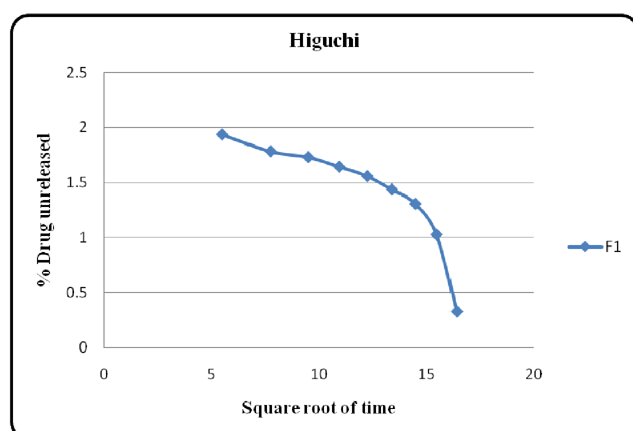

Figure 21: Higuchi model of F1

SKIN IRRITATION STUDY:

FORMULATION CODE	Score	
	DAY-1	DAY-4
Control	0	0
F1	0	0.5

Table 15: Skin irritation study of best optimized gel formulation

Where F1 = Gel formulation 1

0 = No irritation.

0.5 = Faint, barely perceptible and slight dryness.

1 = Defined erythema but no eruption or broken skin.

1.5 = Well defined erythema with dryness and epidermal fissuring.

2 = Moderate erythema.

2.5 = Moderate erythema with barely perceptible edema.

3 = Sever erythema.

3.5 = Moderate to severe erythema with eschar formation.

4 = Moderate to severe erythema with eschar formation and edema extending the applied area.

ANTI FUNGAL STUDIES:

FORMULATION	ZONE OF INHIBITION (mm²)
Standard drug	7.6
Placebo gel	0
F1	6.6

Table 16: Anti fungal studies of optimized gel formulation

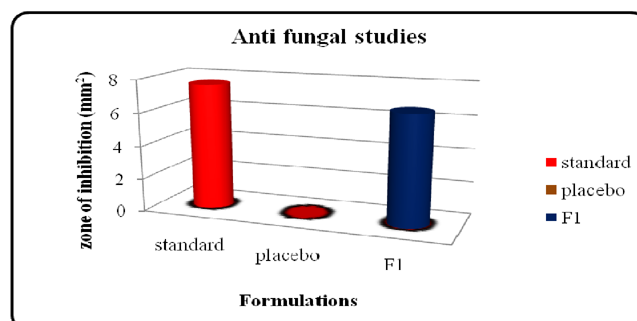

Figure 22: Zone of inhibition of optimized gel formulation

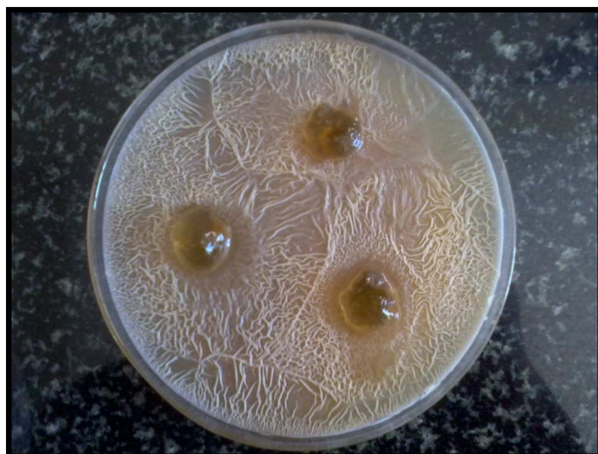

Figure 23: Zone of inhibition of F1 gel formulation

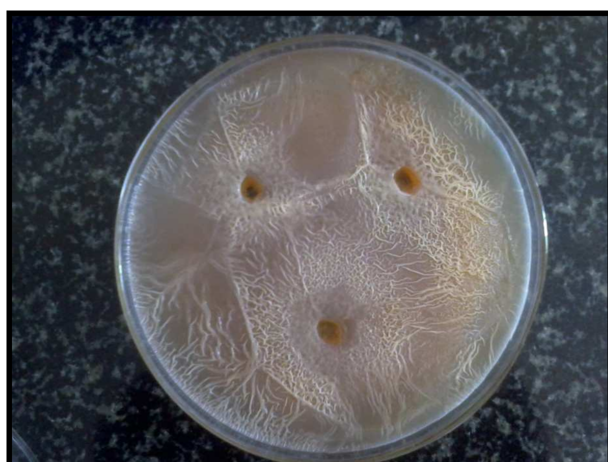

Figure 24: Zone of inhibition of standard gel formulation

Figure 25: Zone of inhibition of Placebo gel formulation

STABILITY STUDIES:

After 1 month:

DRUG CONTENT

FORMULATON	DRUG CONTENT (%)	
	30 ± 2°C at 65 ± 5 RH	40 ± 2°C at 75 ± 5 RH
F1	96.5	75

Table 17: Drug content of optimized gel formulation after 1 month stability studies

IN VITRO DIFFUSION CHART:

TIME (Min)	%CDR	
	F1 A	F1 B
	30 ± 2°C at 65 ± 5 RH	40 ± 2°C at 75 ± 5 RH
0	0	0
30	21	14.49
60	43	18.85
90	50.88	22.55
120	57.46	31.85
150	63.20	35.55
180	72.25	43.36
210	79.60	53.04
240	85.59	61.1
270	97.81	66.60

Table 18: *In vitro* diffusion study of optimized gel formulation after 1 month stability studies

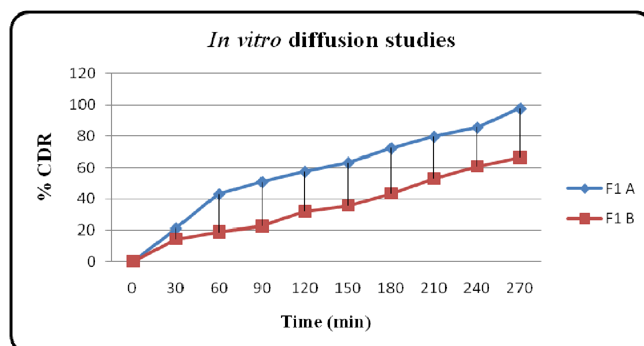

Figure 26: *In vitro* diffusion studies after 1 month stability studies

After 2 month:

DRUG CONTENT

FORMULATON	DRUG CONTENT %	
	30 ± 2°C at 65 ± 5 RH	40 ± 2°C at 75 ± 5 RH
F1	96	6

Table 19: Drug content of optimized gel formulation after 2 months stability studies

IN VITRO DIFFUSION CHART:

TIME (Min)	%CDR	
	F1 A	F1 B
	30 ± 2°C at 65 ± 5 RH	40 ± 2°C at 75 ± 5 RH
0	0	0
30	24.57	0
60	42.33	0
90	48.17	0
120	57.67	0
150	64.26	0
180	73.04	0.21
210	80.85	0.74
240	89.52	1.83
270	97.19	2.54

Table 20: *In vitro* diffusion study of optimized gel formulation after 2 months stability studies

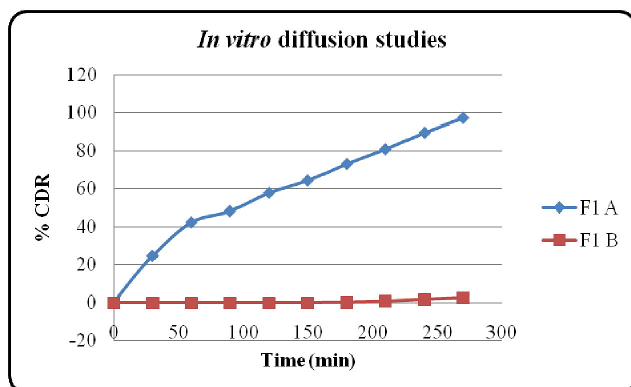

Figure 27: *In vitro* diffusion studies after 2 month stability studies

DISCUSSION

Topical and transdermal drug delivery systems offer several advantages over oral delivery systems. These delivery systems include patch, gel, cream, ointment and lotion. However it has been found so many side-effects were proved by the oral delivery system of fluconazole and here to over the side-effects of oral dosage form. The dosage form has been changed by formulation and evaluation of fluconazole gel.

Fluconazole is an imidazole derivative, used in the treatment of topical as well as systemic fungal infection. The bioavailability of fluconazole is 90%. In the present study, an attempt was made to formulate fluconazole gel for efficient delivery of drug to the skin.

In the present study, fluconazole gel was prepared by using carbopol 934p, alcohol, methyl paraben, propyl paraben, Triethanol amine and distilled water. A total number of five formulations were prepared. The preformulation study of drug-excipients interaction was carried out by FT-IR, which showed no interactions. The data obtained from viscosity studies, drug content, spreadability test, *in vitro* drug diffusion, skin irritation and anti fungal studies gave satisfactory results.

PREFORMULATION STUDIES

Any formulation development work has to be proceeded by Preformulation studies. This Preformulation study includes drug-excipients compatibility study.

FT-IR study showed that there was no major change in the position of peak obtained in the drug alone and in a mixture of drug with excipients, which shows that there was no interaction between drug and excipients.

Estimation of fluconazole was carried out by SHIMADZU-1700 UV spectrophotometer at λ_{max} 260 nm in alcohol. The linear coefficient was found to be r^2= 0.997 which shows that Beer's law obeyed. By using the regression coefficient the %CDR were calculated.

UV Spectrum Analysis of Fluconazole

The method for the estimation for the drug fluconazole showed maximum absorption at wavelength 260 nm in alcohol. Standard curve obeyed Beer's law at given concentration range of 10 µg/ml to 50 µg/ml and when subjected to regression analysis, the value of regression coefficient was found to be 0.997, which showed linear relationship between concentration and absorbance.

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FORMULATION STUDIES

FORMULATION DEVELOPMENT

Various formulation of fluconazole gel was developed using carbopol 934p, alcohol, methyl paraben, propyl paraben, glycerine, Triethanol amine and water. Carbopol 934p was used as polymer; alcohol was used as penetration enhancer; methyl paraben and propyl paraben were used as preservatives; glycerine used as moisturising agent; Triethanol amine used as pH balancer and water used as vehicle.

EVALUATION OF PHYSICOCHEMICAL PARAMETERS

DRUG CONTENT

After various formulation of fluconazole gel the drug content of the formulated gel was estimated by SHIMADZU-1700 UV spectrophotometer at λ_{max} 260 nm in alcohol. The results were in the official limits.

SPREADABILITY

Spreadability test which were carried out for all the formulations, spreadability was of the gel formulation was decreases with the increases in the concentration of the polymer. The spreadability is very much important as show the behaviour of gel comes out from the tube.

***IN VITRO* DRUG DIFFUSION STUDIES**

The release of fluconazole from the gel was varied according to concentration of polymer. The progressive increase in the amount of drug diffusion through a rat skin from formulation F1 attributed to gradual decrease in the concentration of polymer. It has been concluded that, if we increase the concentration of polymer, the diffusion of drug through the skin also decreases. The amount of drug diffused from formulation F1 was 97.846 ± 0.966 in 4½ h which was higher among all the gel formulation.

The order of drug diffused from various formulations was found to decrease in the following order.

F1 > F2 > F3 > F4 > F5

PHARMACOKINETIC PROFILE

The release rate of drug from F1 formulation is best fitted to Higuchi matrix model.

SKIN IRRITATION STUDY

In the skin irritation study no group was used as standard group only two groups was used one for control and another for formulation. The results of skin irritation study revealed no irritation from gel formulation of F1 as it produce a score of 0.5, which was less than 2.

FUNGAL STUDIES

In the anti fungal studies the fungi used was *Candida albicans*. The studies were carried for the best formulation and zone of inhibition observed at F1 (6.6 mm^2), placebo gel (0 mm^2) and pure form of the fluconazole (7.6 mm^2). The results were satisfactory.

STABILITY STUDIES

Stability studies were carried for the most satisfactory formulation-F1, at 30 ± 2°C and 40 ± 2°C at 65 ± 5 and 75 ± 5 RH for 2 months. At the end of 2 months, samples were evaluated.

Drug content study showed that, there was no major change in the content drug of F1 (from 97 ± 0.027 to 96%) at 30 ± 2°C at 65 ± 5 RH and decrease at 40 ± 2°C at 75 ± 5 RH (from 97 ± 0.027 to 6%).

There was no significant change in the *in vitro* drug diffusion study F1 (from 97.846 ± 0.966 to 97.19%) at 30 ± 2°C at 65 ± 5 RH. However, after stability at 40 ± 2°C at 75 ± 5 RH showed decrease in the *in vitro* diffusion study of F1 (from 97 ± 0.027 to 2.54%). This may be due to the effect of temperature on gel-to-liquid transition of lipid bilayers together with possible chemical degradation of the drug.

There was no major in the parameters evaluated like drug content and *in vitro* drug diffusion study of F1 at 30 ± 2°C at 65 ± 5 RH. Thus it can be concluded that, F1 is stable at 30 ± 2°C at 65 ± 5 RH for a period of 2 month

CONCLUSION

- Fluconazole is an imidazole derivative, used for the topical as well as systemic fungal infections. The bioavailability of fluconazole is 90%. In the present study, an attempt was made to formulate topical gel of fluconazole for efficient delivery of drug across the skin.
- A suitable method of analysis of drug by UV spectrophotometry. Fluconazole showed maximum absorption at a wavelength of 260 nm in alcohol. The value of correlation co-efficient was found to be $r^2 = 0.997$, which showed linear relationship between concentration and absorbance. Thus, it can be concluded that, it can be concluded that, beer's law was obeyed.
- Preformulation study for drug-excipients compatibility by FT-IR showed no interaction between drug and selected excipients.
- Various formulation (F1, F2, F3, F4, F5) were developed by using suitable polymer (carbopol 934p) and penetration enhancer.
- Developed formulations of fluconazole were evaluated for the physiochemical parameters such as drug content, viscosity, spreadability, *in vitro* diffusion.
- Viscosity studies of various formulations revealed that formulation F1 was better compare to others.
- Skin irritation study indicated that no irritation have been produced by gel formulation F1.
- Anti fungal studies also showed the good results of formulation F1.
- Viscosity studies of various formulations revealed that formulation F1 was better compare to others.
- From among all the developed formulation, F1 shows better drug diffusion for a period of 4½ h, did not produced skin irritation, good Rheological properties and good results of antifungal studies. Therefore, it was selected as the best formulation.
- The release rate of drug from F1 formulation is best fitted to Higuchi matrix model.
- The most satisfactory formulation-F1 did show any significant change in drug content, *In vitro* drug diffusion studies pattern after stability studies at 30 ± 2°C and at 65 ± 5 RH for 2 months. Thus, the objective of the present work of formulation and evaluating of fluconazole topical gel has been achieved with success.

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