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Formulation Design and *In-vitro* Characterization of Topical Drug Delivery System of Clotrimazole Gel

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A B S T R A C T

The present research was carried out to develop the topical Clotrimazole ethosomal gels. Even though the TDDS faces the problem of drug permeation because of the rigid stratum corneum, it can be overcome by the use of penetration enhancers such as ethanol. The size of the Gel can be reduced by sonication thereby improving the skin permeation properties of gel. By encapsulating Clotrimazole gel the frequency of dosing can be reduced as gel cause the delivery of drug for almost 10hrs. Since the overall drug administered is reduced, the adverse drug reactions of Clotrimazole such as dizziness, allergy, hypotension, etc can also be reduced.

Keywords: Clotrimazole, Ethosomal gels.

A R T I C L E I N F O

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1. Introduction

Optimization of drug delivery through human skin is important in modern therapy. Recently, the transdermal route vied with oral treatment as the most successful innovative research area in drug delivery. Transdermal drug delivery system was first introduced more than 20 years ago. The technology generated tremendous excitement and interest amongst major pharmaceutical companies in the 1980's and 90's. By the mid to late 1990's, the trend of transdermal drug delivery system merged into larger organizations. Transdermal drug delivery system is a type of convenient drug delivery system where drug goes to the systemic circulation through the protective barrier i.e. Skin.

Over the year it has showed promising result in comparison to oral drug delivery system as it eliminates gastrointestinal interferences and first pass metabolism of the drug but the main drawback of TDDS is it encounters the barrier properties of the Stratum Corneum i.e. only the lipophilic drugs having molecular weight < 500 Daltons can pass through it. Ethosomes have been found to be much more efficient in delivering drug to the skin.Transdermal delivery is an important delivery route that delivers precise amount of drug through the skin for systemic action. Discovery of new medicinal agents and related innovation in drug delivery system has not only enabled the successful implementation of novel pharmaceutical, but also permitted the development of new medical treatment with existing drugs. Throughout the past two decades, the transdermal patches have become a proven technology holding the promise that new compound could be delivered in a safe and convenient way through the skin. Since the first transdermal patch was approved in 1981 to prevent nausea and vomiting associated with motion sickness, the FDA has approved through the past 22 years more than 35 transdermal patch products spanning 13 molecules.

Routes of Penetration

Human skin consists of two distinct layers: the stratified avascular cellular epidermis and an underlying dermisof connective tissue. A fatty subcutaneous layer resides beneath the dermis. Hairy skin develops hair follicles and sebaceous glands, and the highly vascularized dermis supports the apocrine and eccrine sweat glands, whichpass through pores in the epidermis to reach the skin surface. With respectto drug permeation, the most important component in this complex membraneis the stratum corneum, or horny layer, which usually provides therate-limiting or slowest step in the penetration process.

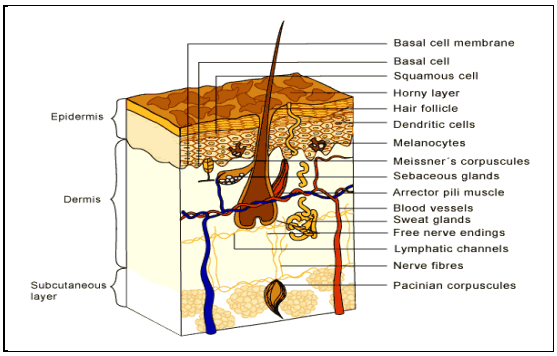


Figure 1: Structure of skin

At the skin molecules contact cellular debris, microorganisms, sebum and other materials, which negligibly affect permeation. The penetration has three potential pathways to the viable tissue.

- Through hair follicles with associated sebaceous glands
- Via sweat ducts
- Or across continuous stratum corneum between these appendages

Vesicular systems are drug delivery system to deliver the drug dermally and transdermally. Liposomes have the potential of overcoming the skin barrier, as these are bilayered lipid vesicles, consisting primarily of phospholipids and cholesterols. Liposomes were discovered in the early 1960's by Bangham and colleagues (Bangham et al., 1965) and subsequently became the most extensively explored drug delivery system. In early 1960's a great knowledge of vesicle derivatives have been tested for their abilities. Most experiments however, were centered on liposomes since derivations only added to their basic properties.

Vesicles are closed, spherical membranes that separate a solvent from the surrounding solvent. Possible use of liposomes in topical drug delivery vehicles for both water and lipid soluble drug has been investigated. While it has been suggested that the external envelop of a liposome would allow it to pass through lipophilic skin, most researches show that liposomal vesicles become trapped within the top layer of the stratum corneum cells. Generally liposomes are not expected to penetrate into viable skin, although occasional transport processes were reported. This behavior is useful both for local treatment of skin disorders and for cosmetic formulations. Specific drug accumulation at the site of action and decreased systemic drug absorption can impart increased efficiency as well as decreased side effect to a compound applied topically.

Factors to be considered when choosing a topical preparation are:

- Effect of the vehicle: An occlusive vehicle enhances penetration of the active ingredient and improves efficacy. The vehicle itself may have a cooling, drying, emollient, orprotective action.
- Match the type of preparation with the type of lesions. For example; avoid greasy ointments for acute weepy dermatitis.
- Match the type of preparation with the site of application. For example; gel or lotion is mostly apply on hairy areas.
- The medication should not affect the skin type.

Formulation of Emulgel

Emulgel formulation contains aqueous phase, oils, and emulsifiers for preparation of emulsion as a vehicle, gelling agents for gel preparation and penetration enhancers to increase the flux of drug through skin.

Vehicle

Emulgel contains emulsion as a vehicle to dissolve drug into it. The vehicle should have following properties for used in topical preparation:

- Efficient and evenly distribution of the drug on the surface of skin.
- Release the drug so easy migration from the site of action.
- Delivery of the drug to the targeted site.
- Sustain a therapeutic drug level in the target tissue for a longer duration to provide a sustained pharmacologic effect .

Emulsifiers

Emulsifying agents are used mainly for promoting emulsification of oil and aqueous phase at the time of manufacture. Emulsifiers retard the phase separation of

emulsion and thus, increasing the stability of emulsion during a shelf life that can vary from days to months or years for commercial preparations

Gelling Agents

Gelling agents used to form gel base to incorporate emulsion in it to prepare emulgel. Gelling agents are the agents which increase the consistency of any dosage form by swelling in aqueous phase and forming gelly like structure. They used as thickening agent in emulgel. The examples of gelling agents are given below.

Penetration Enhancers

Penetration enhancers are the agents which increases the penetration power of the drug through the skin. In order to promote absorption of drugs thorough skin barrier, vehicles often include penetration enhancing ingredients which temporarily disrupts the highly ordered structure of stratum corneum skin barrier, fluidize the lipid channels between corneocytes, alter the partitioning of the drug into skin structures, or otherwise enhance delivery into skin.

Method to Enhance Drug Penetration and Absorption

- Chemical enhancement
- Physical enhancement
- Biochemical enhancement
- Supersaturation enhancement

Method of Preparation of Emulgel

First step involved in emulgel preparation is preparation of emulsion using oil phase and water phase by emulsification method. Drug can be incorporated either in oil or aqueous phase depending upon its solubility. Second step involved preparation of gel using hydrocolloids by soaking in warm water. After cooling, a prepared emulsion is incorporated into preformed gel and stir to uniform disperse emulsion into gel base.

Evaluation Parameters of Emulgel

1. Physical examination
2. Rheological studies
3. Spreading coefficient
4. Extrudability study of topical emulgel (Tube Test)
5. Swelling index
6. Drug Content Determination:
7. Skin Irritation Test (Patch Test)

2. Materials and Methods

Materials:

Table 1: Chemicals and Materials

Chemicals	Manufactured by
Clotrimazole	Natco laboratories (Hyderabad).
Propylene glycol	Research lab fine chem. Industries (Mumbai)
Alcohol	Jiangsu Huaxi International Trade Co. Ltd(CHINA)
Cholesterol	Viratlab (Mumbai).
Carbopol-934	Research lab fine chem. Industries(Mumbai)
Triethanol amine	Research lab fine chem. Industries(Mumbai)
Water	Cortex laboratories (Hyderabad)

Equipments:

Table 2: Instruments and company

Instruments	Company
Electronic weighing balance	Wensar
U.V. spectrophotometer	LABINDIA
Magnetic stirrer	REM elektrotechnik limited. vasai (India)
Refrigerator	Allwyn (INDIA).
Sonicator	SISCO Scientific Instruments sales Corporation, Thana, Mumbai.
pH meter	EI
Scanning electron microscope	Scimadzu corporation (JAPAN).
FTIR	Scimadzu corporation (JAPAN).
Humidity chamber	SISCO Scientific Instruments sales Corporation, Thana, Mumbai

Analytical Methods

Scanning of model drug (Clotrimazole)

10 mg of pure model drug (Clotrimazole) was dissolved in water and was diluted to give concentration of 10µg/ml and was scanned between 220 nm to 300 nm fort the determination of λ_{max}. The wavelength of 234 nm was selected as for λ_{max}. The same was used for further analysis of drug solution and absorbance of final standard solution was also measured at 234 nm.

Preparation of pH 6.8 phosphate buffer: Dissolve 28.8g of disodium hydrogen phosphate and 11.45g of potassium Di hydrogen phosphate in distilled water and then make up the volume to 1000ml with distilled water.

Preparation of calibration curve

10mg of pure Clotrimazole drug was taken in a 10ml standard flask and dissolved in distilled water. The volume of stock solution was made up to 10 ml with pH 6.8 phosphate buffer. From the above stock solution, 1 ml was transferred into a 10ml volumetric flask and volume was adjusted to 10 ml that corresponded to 100µg/ml Clotrimazole in solution. From that solution different aliquots of 0.2, 0.4, 0.6, 0.8, 1.0 and1.2 ml were transferred to 10ml volumetric flask and volume was adjusted to 10ml with ph 6.8 phosphate buffer, which gave a concentration of 2, 4, 6, 8,10 and 12 µg/ml respectively of final standard.

Compatibility Studies

IR spectroscopy can be used to investigate and predict any physicochemical interactions between different components in a formulation and therefore it can be applied to the selection of suitable chemically compatible excipients. The aim of present study was to test whether there is any interaction between the carriers and the drug.

The IR spectroscopy was recorded for following compounds.

- model drug (Clotrimazole)
- Clotrimazole emulgel

One part of the sample and three parts of potassium

bromide were taken in a mortar and triturated. A small amount of triturated sample was taken into a pellet maker and was compressed at 10kg/cm^2 using hydraulic press. The pellet was kept on to the sample holder and scanned from 4000cm^{-1} to 625cm^{-1} (about 2.5µm to 16µm) using Bruker IR spectrophotometer. Then it was compared with original spectra. IR spectra was compared and checked for any shifting in functional peaks and non-involvement of functional group.

Preparation of Clotrimazole (by Cold Method)

Preparation of Clotrimazole was followed by method. The Gel system of Clotrimazole comprised of

- 2-5% phospholipids (soy lecithin),
- 20-50% ethanol,
- 10% of propylene glycol,
- 0.005g of cholesterol
- aqueous phase to 100% w/w

100mg of Clotrimazole was dissolved in 6ml of water in a vessel and cholesterol was added to it with vigorous stirring. Propylene glycol was also added during stirring. The contents were heated to 30^{0}c. In another closed vessel, soy lecithin was dissolved in ethanol with continuous stirring and heated to 30^0 C. When both the solutions reached to same temperature slowly ethanol solution was added drop wise in the centre of vessel containing drug mixture. Then the stirring was continued for another 10min in a covered vessel. Water was added to adjust the volume up to 20 ml.

Characterization of Ethosomes

Size and shape analysis

Microscopic analysis was performed to determine the average size of particles. A sample of gel was suitably diluted with distilled water in order to observe individual vesicle and a drop of diluted suspension was put on a glass slide covered with a cover slip. This was examined under microscope (magnification 15 × 45 X). The diameter of 150 vesicles was determined randomly using calibrated eyepiece micrometer with stage micrometer. The average diameter was calculated using the following formula.

$$\text{AverageDiameter } (d_{avg}) = \frac{nd}{n}$$

Where

n = number of vesicles

d = diameter of vesicles

Sonication reduced the vesicular size. Since the vesicular size of these vesicles could not be analyzed using microscopic method at magnification 15×45X. Hence analysis of sonicated vesicles was done under a special microscope which was connected with software and photomicrographs were taken under 400 and 800 magnification.

Scanning Electron Microscopy (SEM)

Determination of surface morphology (roundness, smoothness and formation of aggregates) of Clotrimazole gel with polymer was carried out by using scanning electron microscopy (SEM).

Entrapment efficiency[11]

The entrapment efficiency of Clotrimazole dug into vesicle was determined by using ultracentrifugation. 10 ml (Clotrimazole) of each sample was vortexed for 2 cycles of 5 min with 2 minutes rest between the cycles. 1.5ml of each vortexed sample and fresh untreated Gel formulations were taken into different centrifugal tubes. These samples were centrifuged at 20,000 rpm for 3 hours. The supernatant layer was separated, diluted with water suitably and drug concentration was determined at 234 nm in both vortexed and unvortexed samples. The entrapment efficiency was calculated as follows

$$\text{Entrapment Efficiency} = \frac{T - C}{T} \text{ x } 100$$

Where

T = Total amount of drug that was detected from supernatant of vortexed formulation

C = Amount of drug unentrapped and detected from supernatant of unvortexed formulation

Characterization of Gel

Surface morphology

The surface morphology of the gel was determined by scanning electron microscope using gold sputter technique. The system was vacuum dried, coated with gold palladium, and then observed microscopically.

Organoleptic Characters

The formulations were tested for their psycho rheological properties like color, odor, texture, phase separation and feel upon application (grittiness, greasiness)

Washability

A small quantity of gel was applied on the skin. After washing with water, it was checked whether the gel was completely washable or not.

Spreadability

It was determined by using modified wooden block and glass slide apparatus. A measured amount of gel was placed on fixed glass slide; the movable pan with a glass slide attached to it was placed over the fixed glass slide such that the gel was sandwiched between the two glass slides for 5min. The weight was continuously removed. Spreadability was determined using the below formula.

$$S = \frac{M}{T}$$

Where,

S = Spreadability in g/s

M = Mass in grams

T = Time in seconds

pH measurement

Solution was prepared by dissolving 1gm of Clotrimazole gel in 30ml of distilled water (pH 7). The pH of gel was determined by using digital pH meter. The measurement was done by bringing the probe of the pH meter in contact with the samples.

Drug content and content uniformity

1g of gel was dissolved in 100mlof phosphate buffer (pH 6.8) and kept for 48 hrs with constant stirring using magnetic stirrer. Then the solution was filtered and the absorbance was observed using U.V spectrophotometer at λmax i.e. 234nm. The measurements were made in triplicate.

Skin irritation

Rat (male Wristar rat) was taken and the abdominal skin of the rat was clipped free of hair 24 hr prior to the formulation application. 0.5 g of each formulation was

applied on the hair-free skin of rat by uniform spreading over an area of 4 cm^2. The skin surface was observed for any visible change such as erythema (redness) after 24, 48 and 72 hr of the formulation application. The mean erythemal scores were recorded depending on the degree of erythema.

No erythema = 0,
Slight erythema (barely perceptible- light pink) = 1,
Moderate erythema (dark pink) = 2,
Moderate to severe erythema (light red) = 3 and
Severe erythema (extreme redness) = 4.

In-Vitro Release Studies

Drug Release Study from Dialysis Membrane

The skin permeation of Clotrimazole from gel formulation was studied by using an open ended diffusion cell specially designed laboratory according to the literates. The effective permeation area of the diffusion cell and receptor cell volume was 2.4 cm and 200 ml respectively. The temperature was maintained at 37 ± 0.5°C. The receptor compartment contained 200 ml of pH 6.8 phosphate buffer and was constantly stirred by magnetic stirrer at 100 rpm. The dialysis was prepared by using semi permeable membrane from egg. The membrane was tied to an open end tube. This served as the donor compartment where as the beaker containing phosphate buffer served as the receptor compartment. Gel formulation F_1-F_7 (20ml suspension) and for optimized gel (10gm)] was applied to the dialysis membrane and the content of diffusion cell was kept under constant stirring. Then 5 ml of samples were withdrawn from receptor compartment of diffusion cell at predetermined time intervals and analyzed by spectrometric method at 234 nm after suitable dilution. The receptor phase was immediately replenished with equal volume of fresh pH 6.8 buffer. Triplicate experiments were conducted for drug release studies.

In-vitro release kinetics:

To analyze the in vitro release data, various kinetic models were used to describe the release kinetics. The zero order rate equation describes the systems where the drug release rate is independent of its concentration. The first order rate equation describes the release from system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion. The results of in vitro release profile obtained for all the formulations were plotted in modes of data treatment as follows:

- Zero -order kinetic model– Cumulative percentage drug released versus time.
- First–order kinetic model– Log cumulative percent drug remaining versus time.
- Higuchi's model– Cumulative percent drug released versus square root of time.
- Kerseymere equation/ Peppa's model–Log cumulative percent drug released versus log time.

Stability Studies

Stability study was carried out for Clotrimazole Gel preparation at two different temperature i.e. refrigeration temperature (4 ± 2° C) and at room temperature (27 ± 2° C) for 8 weeks (as per ICH guidelines). The formulation was subjected to stability study and stored in borosilicate container to avoid any sort of interaction between the Gel preparation and glass of container, which may affect the observations.

In-vitro stability release study

Stability of drug and stability of vesicles are the major determinant for the stability of formulation. Studies were carried to evaluate total drug content at room temperature (27±2° C) and at refrigeration temperature (4±2° C). Samples were collected for every 2 weeks and absorbance was seen at 234nm in U.V spectrometer.

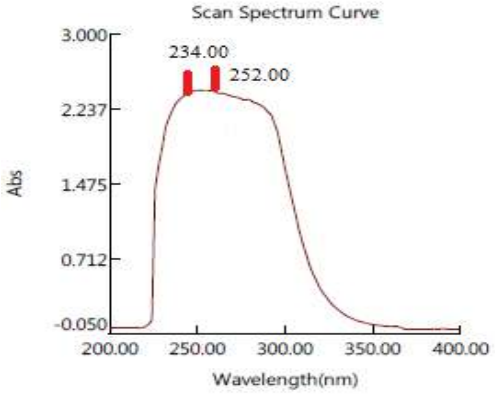
3. Results and Discussions

Scanning of drug

Clotrimazole pure drug was scanned in methanol between 200 nm and 400 nm using ultraviolet spectrophotometer. Clotrimazole was identified by its light absorption pattern which follows the absorption of light in the range 220 to 360 nm and a maximum absorbance at about 234 nm. A broad shoulder at about 234 nm was observed which confirm the presence of Clotrimazole. Clotrimazole gave highest peak at 234 nm and the same was selected for further evaluations.

Calibration curve in water (make up with ph 6.8 phosphate buffer)

Standard solutions of different concentrations were prepared and their absorbance was measured at 234 nm. Calibration curve was plotted against drug concentrations versus absorbance as given in the (Figure.).



Comment :

No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	234.00	2.567	
2	Peak	252.00	2.545	

Figure 2: Clotrimazole absorbance spectrum

Table 3: Determination of λ_{max} of Clotrimazole in methanol-- λ_{max} = 234 nm

Concentration (µg /ml)	Absorbance
0	0
2	0.14
4	0.269
6	0.383
8	0.496
10	0.618
12	0.740

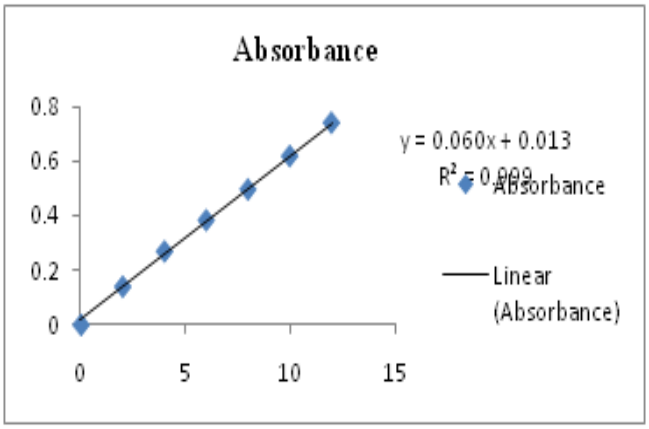


Figure 3: Standard graph of Clotrimazole

Preparation of Clotrimazole (by Cold Method)

Gel formulations composed of phospholipids (lecithin, cholesterol), Clotrimazole and ethanol were prepared using the method detailed in last chapter titled materials and methods and also according to the literature with little modification in it. gel suspension was slight yellowish in color and hazy in appearance after sonication. Different characteristics of and the effect of sonication were further evaluated and results were reported under characterization.

Characterizations

Since the physical characterization is meant for physical integrity of the dosage form, the results were pooled at one place. Discussion on the results, described for gel formulation under the same heading.

Size and shape analysis

Microscopic analysis was performed under different magnification to visualize the vesicular structure, lamellarity and to determine the size of gel preparations. The size distribution of Clotrimazole gel formulations were as shown below

Entrapment Efficiency

Once the presence of bilayer vesicles was confirmed in the gel system, the ability of vesicles for entrapment of drug was investigated by ultra centrifugation. Ultra-centrifugation was the method used to separate the gel vesicles containing drug and un-entrapped or free drug, to find out the entrapment efficiency. The maximum entrapment efficiency of gel vesicles as determined by ultracentrifugation was 79.62% for gel formulation containing 30% ethanol (F5) which was almost double to the formulation containing 50% ethanol (F6). As the ethanol concentration increased from 20% to 50% w/w, there was an increase in the entrapment efficiency and with further increase in the ethanol concentration (>30% w/w) the vesicle membrane became more permeable and that lead to decrease in the entrapment efficiency.

Results of entrapment efficiency also suggest that 3% phospholipid concentration is optimum for entrapment efficiency. Any increase or decrease in concentration of phospholipid reduces the entrapment efficiency of vesicles. These result further supported the observation made by Jain NK et al., [7] Increase in entrapment efficiency may be due to the possible reduction in vesicle size. There is a detrimental effect on the vesicles during ultra-centrifugation which are larger in size. Sonication gives more uniform lamellae with smaller vesicle and uniform size. Hence it may be the reason for higher vesicular stability and lesser vesicular disruption during ultra centrifugation

***In-Vitro* Drug Permeation Studies**

In-vitro skin permeation study or in-vitro diffusion study has been extensively studied, developed and used as an indirect measurement of drug solubility, especially in preliminary assessment of formulation factors and manufacturing methods that are likely to influence bioavailability. The objectives in the development of in-vitro diffusion tests are to show the release rate and extent of drug from the dosage form.

The in-vitro drug permeation study of Clotrimazole from gel formulation was studied using Franz diffusion cell and the method described in methodology chapter. The release data was obtained for all the gel formulations. Spectrometric results were obtained and given consideration to sampling loss, to calculate actual cumulative drug diffused was calculated since the volume of receptor cell was only 20 ml (table-24). The obtained diffused amount of drug was extrapolated to diffusion by unit surface area of semi permeable membrane. These cumulative values were plotted as a function of time and steady state transdermal flux was calculated from the slop of linear portion.

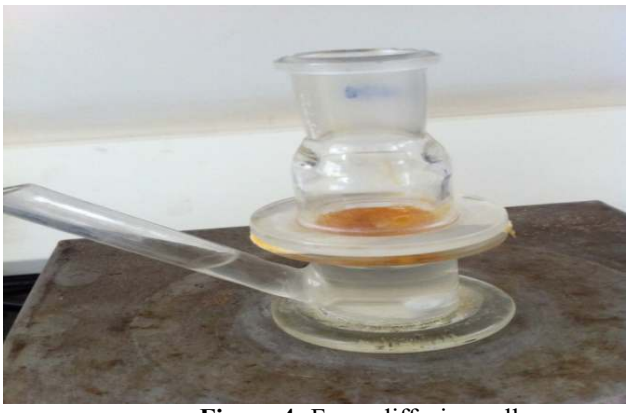


Figure 4: Franz diffusion cell

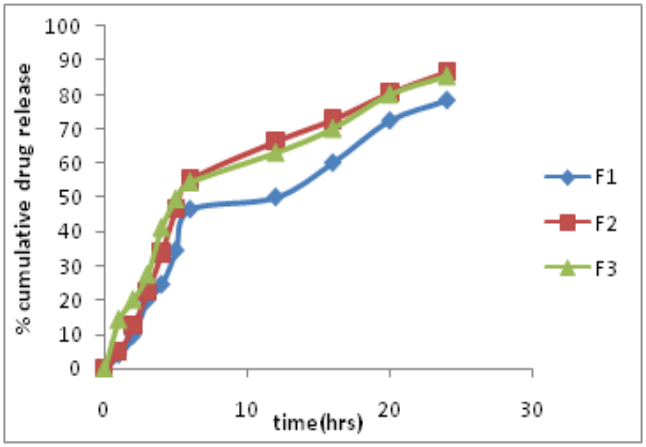


Figure 5 A: *In-vitro* drug release studies of different gel formulations

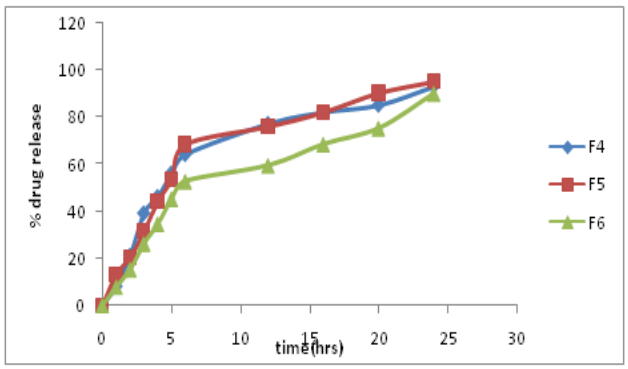


Figure 5B: In-vitro drug release studies of different gel formulations

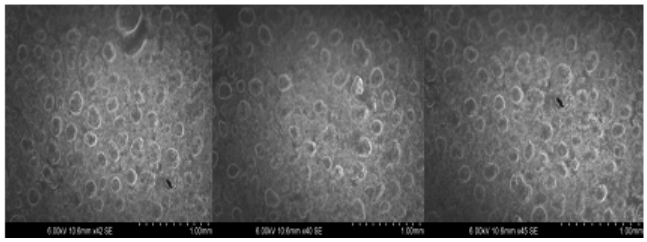


Figure 6: SEM Results

FTIR Spectrum:

Figure 7A: FTIR Spectrums of Pure Drug and Optimized Formulation

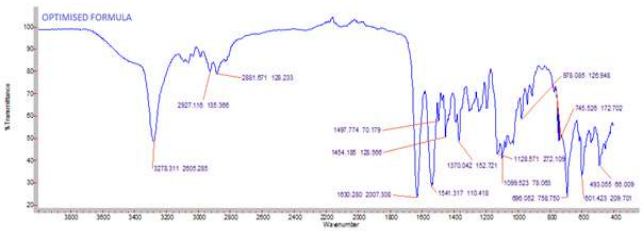


Figure 7B: FTIR Spectrums of Pure Drug and Optimized Formulation

The FTIR Spectrum of clotrimazole formulation showed absorption band at 3064.99 cm-1 (C-H, str.), 1494.88 cm-1 (C-C, str. In aromatic ring), 1442.80 cm-1 (C-N, str. In aromatic ring), 765.77, 752.26 cm-1 (Aromatic C-H, out of plane bending), 707.90, 696.33, 673.18 cm-1 (Aromatic ring C-H, out of plane bending), it is near to FTIR of pure clotrimazole so, drug and excipients were compatible.

Observation:

By observing the above spectrums it was concluded that there is no change in the appearance of the characteristic peaks of the pure drug and polymer it shows the compatability between the components of formulation.

4. Conclusion

From the present study it can be concluded that Clotrimazole gel is promising route of drug administration. Even though the TDDS faces the problem of drug permeation because of the rigid stratum corneum, it can be overcome by the use of penetration enhancers such as ethanol. The size of the Gel can be reduced by sonication thereby improving the skin permeation properties of gel. By encapsulating Clotrimazole gel the frequency of dosing can be reduced as gel cause the delivery of drug for almost 10hrs. Since the overall drug administered is reduced, the adverse drug reactions of Clotrimazole such as dizziness, allergy, hypotension, etc can also be reduced.

Table 4: Transmission minima and acceptable tolerances of a polystyrene film

Transmission minima (cm^{-1})	Acceptable tolerance (cm^{-1})	
	Monochromater instruments	FTIR instruments
3060.0	±1.5	±1.0
2849.5	±2.0	±1.0
1942.9	±1.5	±1.0
1601.2	±1.0	±1.0
1583.0	±1.0	±1.0
1154.5	±1.0	±1.0
1028.3	±1.0	±1.0

Table 5: Composition of different emulgel formulations

Formulation (F)	Lecithin (%)	Propylene Glycol (%)	Ethanol (%)	Cholesterol (mg)	Drug (mg)	Water
F_1	2	10	20	0.05	100	Q.s
F_2	3	10	20	0.05	100	Q.s
F_3	4	10	20	0.05	100	Q.s
F_4	3	10	30	0.05	100	Q.s
F_5	**3**	**10**	**40**	**0.05**	**100**	**Q.s**

F_6	3	10	50	0.05	100	Q.s

Table 6: Size distribution of gel formulation#1 F_1 (2% Lecithin, 20% ethanol)

Size Range					
Eye piece micrometer	**In µm**	**Average size (d) µm**	**No of vesicles (n)**	**% No of vesicles (n/150 *100)**	**n x d**
0-1	0.00-3.33	1.665	65	43.33	108.225
1-2	3.33-6.66	4.995	62	41.33	309.69
2-3	6.66-9.99	8.325	11	7.33	91.575
3-4	9.99-13.32	11.655	7	4.667	81.585
4-5	13.32-16.65	14.985	5	3.33	74.925
			Σn = 150		**Σnd = 666**

$$\textbf{Average diameter (d avg)} = \frac{\Sigma nd}{\Sigma n} = \textbf{4.44 µm}$$

Table 7: Size distribution of gel formulation#2 F_2 (3% Lecithin, 20% ethanol)

Size Range					
Eye piece micrometer	**In µm**	**Average size (d) µm**	**No of vesicles (n)**	**% No of vesicles (n/150 *100)**	**n x d**
0-1	0.00-3.33	1.665	60	40.000	99.9
1-2	3.33-6.66	4.995	45	30.000	224.775
2-3	6.66-9.99	8.325	30	20.000	249.75
3-4	9.99-13.32	11.655	10	6.667	116.55
4-5	13.32-16.65	14.985	5	3.333	74.925
			Σn = 150		**Σnd = 765.9**

$$\textbf{Average diameter (d avg)} = \frac{\Sigma nd}{\Sigma n} = \textbf{5.106 µm}$$

Table 8: Size distribution of gel formulation#3 F_3 (4% Lecithin, 20% ethanol)

Size Range					
Eye piece micrometer	**In µm**	**Average size (d) µm**	**No of vesicles (n)**	**% No of vesicles (n/150 *100)**	**n x d**
0-1	0.00-3.33	1.665	58	38.667	96.57
1-2	3.33-6.66	4.995	40	26.667	199.8
2-3	6.66-9.99	8.325	27	18.000	224.775
3-4	9.99-13.32	11.655	22	14.667	256.41
4-5	13.32-16.65	14.985	3	2.000	44.955
			Σn = 150		**Σnd = 822.51**

$$\textbf{Average diameter (d avg)} = \frac{\Sigma nd}{\Sigma n} = \textbf{5.483 µm}$$

Table 9: Size distribution of gel formulation#4 F_4 (3% Lecithin, 30% ethanol)

Size Range					
Eye piece micrometer	**In µm**	**Average size (d) µm**	**No of vesicles (n)**	**% No of vesicles (n/150 *100)**	**n x d**
0-1	0.00-3.33	1.665	59	39.333	98.235
1-2	3.33-6.66	4.995	48	32.000	239.76
2-3	6.66-9.99	8.325	26	17.333	216.45
3-4	9.99-13.32	11.655	15	10.000	174.825
4-5	13.32-16.65	14.985	2	1.333	29.97
			Σn = 150		**Σnd = 765.9**

$$\textbf{Average diameter (d avg)} = \frac{\Sigma nd}{\Sigma n} = \textbf{5.062 µm}$$

Table 10: Size distribution of gel formulation#5 F_5 (3% Lecithin, 40% ethanol)

Size range					
Eye piece micrometer	**In µm**	**Average size (d) µm**	**No of vesicles (n)**	**% No of vesicles (n/150 *100)**	**n x d**
0-1	0.00-3.33	1.665	64	42.667	106.56
1-2	3.33-6.66	4.995	52	34.667	259.74
2-3	6.66-9.99	8.325	23	15.333	191.475
3-4	9.99-13.32	11.655	11	7.333	128.205
4-5	13.32-16.65	14.985	2	1.333	29.97
			Σn = 150		**Σnd = 715.95**

$$\textbf{Average diameter (d avg)} = \frac{\Sigma nd}{\Sigma n} = 4.71\ \mu m$$

Table 11: Size distribution of gel formulation#6 F_6 (3% Lecithin, 50% ethanol)

Size Range					
Eye piece micrometer	**In µm**	**Average size (d) µm**	**No of vesicles (n)**	**% No of vesicles (n/150 *100)**	**n x d**
0-1	0.00-3.33	1.665	70	46.667	116.55
1-2	3.33-6.66	4.995	67	44.667	334.665
2-3	6.66-9.99	8.325	7	4.667	58.275
3-4	9.99-13.32	11.655	4	2.667	46.62
4-5	13.32-16.65	14.985	2	1.333	29.97
			Σn = 150		**Σnd =586.08**

$$\textbf{Average diameter (d avg)} = \frac{\Sigma nd}{\Sigma n} = 3.907\mu m$$

Table 12: Drug entrapment efficiency of Clotrimazole Gel

Formulation code	**Entrapment efficiency (%)**			**MEAN**
F1	72.19	71.75	71.82	71.92
F2	66.91	67.12	68.53	67.52
F3	60.05	60.00	60.01	60.02
F4	58.01	55.96	54.96	56.31
F5	**79.91**	**79.62**	**79.33**	**79.62**
F6	39.39	42.32	42.76	41.49

Table 13: Organoleptic characters of Clotrimazole gel

Organoleptic Characteristics	Color: golden yellow Greasiness: Non greasy Grittiness: Free from grittiness Ease of application: Easily/smoothly applied Skin irritation: No skin irritation
Washability	Easily washable without leaving any residue on the surface of the skin.
Spreadability	6.25 cm/sec

The pH of gel was measured by using electrode based digital pH meter.

Table 14: *In-vitro* cumulative % drug release profile for Clotrimazole

Cumulative % drug release						
Time	F_1	F_2	F_3	F_4	F_5	F_6
0	0	0	0	0	0	0
1	4.02	4.78	14.25	8.22	13.21	7.85
2	9.54	12.47	20.11	21.25	20.25	15.25
3	19.88	22.35	27.45	39.25	31.58	26.24
4	24.58	33.65	41.22	46.21	44.21	34.58
5	34.55	46.51	49.58	56.21	53.75	45.31
6	46.58	55.12	54.55	64.22	68.55	52.74
12	50.14	66.32	63.22	77.25	76.21	59.54

16	60.21	72.67	70.25	82.21	82.31	68.54
20	72.55	80.45	80.22	85.21	90.55	75.34
24	78.54	86.54	85.64	93.21	95.32	90.21

Table 15: Stability studies for optimised formulation (F5)

S.No	Optimised formulation (F5) duration	25^0C (75%RH)	37^0C (75%RH)
1	1 MONTH	95.30%	95.10%
2	2 MONTH	95.27%	94.80%
3	3MONTH	95.20%	94.75%

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