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FORMULATION AND EVALUATION OF HYDROGEL CONTAINING MICRO EMULSION OF AMPHOTERICIN-B AND FLUCONAZOLE

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ABSTRACT: The formulation and evaluation of a hydrogel containing a microemulsion of Amphotericin B and Fluconazole represent a novel approach in drug delivery systems. This study aimed to develop a hydrogel formulation that incorporates a microemulsion of Amphotericin B and Fluconazole for enhanced therapeutic efficacy. The hydrogel was prepared using suitable polymers and characterized for various parameters like pH, viscosity, drug content, spreadability, and in vitro release studies. The evaluation of the hydrogel demonstrated promising results in terms of drug content uniformity, sustained release profile, and stability. The developed hydrogel showed potential for controlled drug delivery with improved bioavailability and reduced dosing frequency.

KEYWORDS: Formulation, Hydrogel, Micro-emulsion, Amphotericin- b, Fluconazole, NDDS.

INTRODUCTION: Over the last decades the treatment of illness has been accomplished by administrating drugs to human body via various routes namely oral, sublingual, rectal, parental, topical, inhalation etc. Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin.

1.1 Fungal infections

Fungal infections are any disease or condition you get from a fungus. They usually affect your skin, hair, nails or mucous membranes but they can also infect your lungs or other parts of your body.

- **Superficial fungal Infections**

Superficial fungal infections affect your nails, skin and mucous membranes (like your mouth, throat or vagina).

Ringworm (dermatophytosis). A group of fungi that live off of skin, hair and nail cells (dermatophytes) cause ringworm. They can infect your feet (tinea pedis/athlete's foot), your groin and inner thighs (tinea cruris/jock itch), your scalp (tinea capitis), your hands (tinea manuum), your facial hair and skin around it (tinea barbae) and other parts of your body (tinea corporis)

Onychomycosis. Many types of fungi cause infections of your fingernails or toenails (onychomycosis). This can cause discolored and cracked nails.

Deep or invasive fungal infections include:

Histoplasmosis. Histoplasma, the fungus that causes histoplasmosis, can infect your lungs, brain or other parts of your body. It's commonly found in the Ohio and Mississippi River valleys.

Coccidioidomycosis (Valley fever). Caused by the fungus Coccidioides, coccidioidomycosis can infect your lungs and, rarely, move to other parts of your body. It's most common in California and Arizona.

1.2 Microemulsions

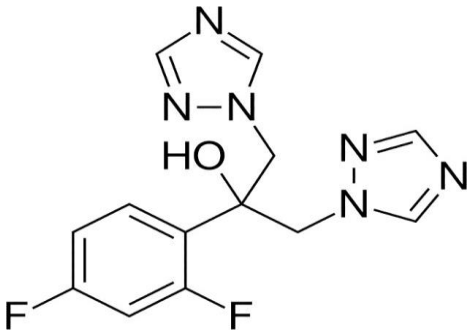
Microemulsions are isotropic transparent liquid systems of water, oil, and amphiphile. ME systems exist in three different forms depending on the components of the ME with the water content playing an integral role in determination of the phase behavior of the developed systems. Three types of MEs have been identified as oil-in-water (O/W ME) in which water represents the continuous phase; water-in-oil (W/O ME), which has the aqueous phase as the internal phase; and bicontinuous water and oil ME, which comprises comparable amounts of the aqueous and oily phases. MEs are characterized by thermodynamic stability. MEs can be developed by mixing the oily phase with water in the presence of suitable surfactant/cosurfactant system. The relative proportions of these components affect the phase behavior of the developed system with those having low surface tension showing spontaneous transformation into ME.

1.3 Hydrogel

A hydrogel is a biphasic material, a mixture of porous, permeable solids and at least 10% by weight or volume of interstitial fluid composed completely or mainly by water. In hydrogels the porous permeable solid is a water insoluble three dimensional network of natural or synthetic polymers and a fluid, having absorbed a large amount of water or biological fluids.These properties underpin several applications, especially in the biomedical area. Many hydrogels are synthetic, but some are derived from nature. The term 'hydrogel' was coined in 1894.

1.4 Fluconazole

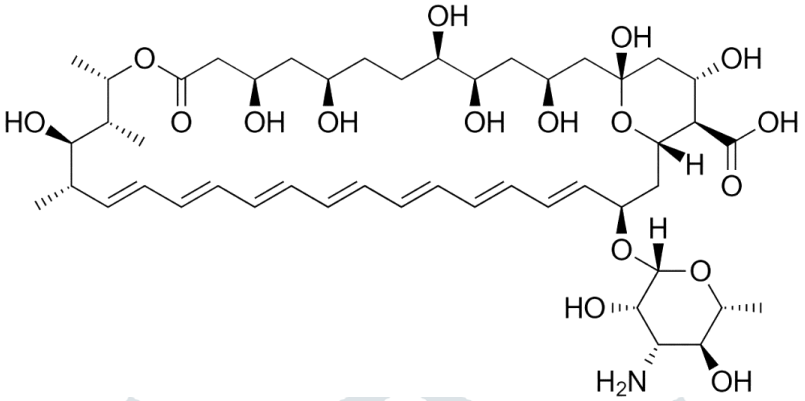
Fluconazole is an antifungal medication used for a number of fungal infections. This includes candidiasis, blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, dermatophytosis, and tinea versicolor. It is also used to prevent candidiasis in those who are at high risk such as following organ transplantation, low birth weight babies, and those with low blood neutrophil counts. It is given either by mouth or by injection into a vein. The risk of miscarriage while large doses may cause birth defects. Fluconazole is in the azole antifungal family of medication. It is believed to work by affecting the fungal cellular membrane.



1.5 Amphotericin B

Amphotericin B is an antifungal medication used for serious fungal infections and leishmaniasis. The fungal infections it is used to treat include mucormycosis, aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, and cryptococcosis. For certain infections it is given with flucytosine. It is typically given intravenously (injection into a vein).

Common side effects include a reaction with fever, chills, and headaches soon after the medication is given, as well as kidney problems. Allergic symptoms including anaphylaxis may occur. Other serious side effects include low blood potassium and myocarditis (inflammation of the heart). It appears to be relatively safe in pregnancy. There is a lipid formulation that has a lower risk of side effects. It is in the polyene class of medications and works in part by interfering with the cell membrane of the fungus.



1.6 Topical delivery

It includes two basic types of products

- External topicals that are spread, sprayed, or otherwise dispersed on to cutaneous tissues to cover the affected area.
- Internal topicals that are applied to the mucous membrane orally, vaginally or on anorectal tissues for local activity.

Advantages of Topical Drug Delivery Systems

- Avoidance of first pass metabolism.
- Convenient and easy to apply.
- Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time etc.
- Achievement of efficacy with
- lower total daily dosage of drug by continuous drug input.
- Avoids fluctuation in drug levels, inter- and intrapatient variations.
- Ability to easily terminate the medications, when needed

Permeation through skin

Most of topical preparations are meant to be applied to the skin. So basic knowledge of skin and its physiology, function and biochemistry is very important for designing topicals. The skin is the heaviest single organ of the body, combines with the mucosal lining of the respiratory, digestive and urogenital tracts to from a capsule, which separates the internal body structures from the external environment. The pH of the skin varies from 4 to 5.6. Sweat and fatty acids secreted

from sebum influence the pH of the skin surface. It is suggested that acidity of the skin helps in limiting or preventing the growth of pathogens and other organisms.

MATERIAL METHOD:

The micro emulsion based hydrogel is composed of mainly three ingredients; oleic acid. oween 80, and carbopol 940. Usually it is made up of two phases, first oleic acid and cween 50 (oil phase) and second water and carbopol 940 (aqueous phase), i.e. hydrogel is combine with oil phase made up of oleic acid

and cween 80.

For the preparation of hydrogel firstly we take carbopol and kept it 4 hrs for swelling then stan the procedure of formulation. For preparation of hydrogel I used the drug as active ingredient, carbopol-940 as gelling agent and other excipients were used. Firstly i added 15ml of oleic acid with 9.5ml of cween 80 with continuous stirring at room temperature on 500 rpm. Then after 1 hr of continuous stirring added 10 mg of Amphotericin-B in it. Then again wait for 1 hr and now added 4ml of water (drop by drop) with 50 mg of Fluconazole. Then added carbopol-940 that had been swelled from last 4 hrs now leave it for another 3 to 4 hr on magnetic stirrer and kept it aside for 24 hr for proper gel formation. Now hydrogel containing micro emulsion of Amphotericin-B and Fluconazole is ready.

Evaluation Parameters

1) Physical Evaluation: Gels were visually checked for colour, odour, consistency and homogeneity.
2) Ph Measurement: The pH of prepared gels was determined using a digital pH meter, which was calibrated before each use with standard pH solution. Each formulation was found in an oral cavity pH range (6.8-7.2).
3) Viscosity: Viscosities of all 8 formulated gels were measured by using Brookfield viscometer at 100 rpm using spindle number 64. Viscosities were recorded at room temperature for all formulations.
4) Spreadibility: Two equal sized glass plates were taken and about 1 gm of gel was placed into a circle of 1 cm diameter marked on a graph paper which was placed billow a glass plate, over which a second glass plate was placed. A weight of 100 g was allowed to rest on the upper glass plate and increase in a diameter due to the spreading of the gels was noted. Spreadability was determined using following formula.
5) S=ML/T

5)Drug Release Study: An in-vitro drug release study was carried out using Franz's Diffusion cell (Dolphin) and egg membrane. An egg membrane was stored in phosphate buffer (pH 6.8) for 24 hrs before use. Egg membrane was tied to one end of donor compartment and the receptor compartment was filled with the phosphate buffer of 6.8 pH and temperature was maintained at 37 ±0.5°C with constant stirring. Igm of gel was placed on a donor compartment. The Iml samples was collected from the receptor compartment at predetermined time interval and replaced by equal volume of phosphate buffer to maintain a sink condition throughout the experiment. The amounts of drugs in the sample were assayed by using UV-Vis spectrophotometer (Shimadzu-1900) at 210 and 410 nm.

1. **Preformulation studies**

Preformulation studies performed before the commencement of formulation development,and the major aim of the study is to produce or develop stable, safe, and therapeutically effective and efficacious dosage forms that are mainly related to the characterization of the physico-chemical properties of the drug molecule**.**

2. **Identification of drug**

FT-IR spectroscopy method was used for the identification and evaluation of drug and excipients. Drug KBR pellets were used to record the FT-IR spectrum with a Perkin-Elmer model.

Process – 3: - Analysis

The content for Amphoteracin-B and fluconazole in microemulsion was estimated by UV spectrophotometer technique which is based on the measurement of absorbance at wavelength 275 nm inphosphate buffer medium at pH 7.4. The technique was validated for its accuracy andprecision. The method obeyed Beer's law in the concentration range 0-25 µg/ml. In observation (n=6), the mean error (accuracy) and relative S.D. (precision) were found to be 0.6% & 1.2% respectively.

3. In vitro evaluation techniques

3.1 In-vitro diffusion studies

Skin (abdomen) of swiss albino male mice was taken for diffusion procedures. Mice (30-35g) were anesthetized slightly by di-ethyl-ether and hairs were removed from the skin of mice. They were sacrificed and the abdominal skin of mice was taken off. After removing the subcutaneous fat the skin was washed and checked for its integrity. The skin was stored in a refrigerator at 4°C overnight and then used for the evaluation. The diffusion procedures were performed in a diffusion cell with a recirculating water bath with 12 diffusion cells. The skin was stretched and fixed between the donor and the receptor chamber of diffusion cells. The cell has an effective diffusion area of 2.8 cm^2 and 7 ml volume of cell. The receptor chamber was filled with freshly prepared mixture of water ethanol in the ratio of 4:1 v/v to solubilize amphoteracin-B and fluconazole. The solution of 20% ethanol was used to solubilize amphoteracin-B and fluconazole. The receptor chambers were thermostat at 37°C and the solution in the receptor chambers was stirred (continuously) at 300 rpm. The formulation (1.5 g) containing amphoteracin-B and fluconazole was kept in the donorchamber. At appropriate time interval, 0.5 ml of the solution from receptor chamber was removed for UV evaluations and replaced immediately with the same volume of fresh solution of ethanol (20%). The cumulative amount of drug diffused through mice skin was plotted against time (Salgado et al. 2010)

1.1 Skin irritation studies

A set of 8 rats was used for studying skin irritation test. The Emulgel was applied on the shaven skin of rats. The undesirable skin changes i.e., change in colour, scratches and change in morphology were determined within 24 hours of application.

Table 1: Spectrum of activity for antifungal drugs

	Fluconazole	Amphotericin B
C. albicans	++	++
C. dubliniensis	++	++
C. tropicalis	++	++
C. glabrata	+/-	++
C. krusei	-	++
C. parapsilosis	++	++
C. guilliermondii	+	++
C. lusitaniae	++	-

Stability of microemulsions

Amphoteracin-B and fluconazole microemulsion was evaluated for its physical and chemical characteristics via phaseseparation employing mechanical stress study and residual drug content. Microemulsions were stored at 8°C, 45°C, and 60°C for 6 months. Then these parameters were evaluated to assure the optimum storage conditions for the Amphoteracin- B and fluconazole microemulsion.

S No.	Time (min)	Percent drug release of Amphotericin-B	Percent Drug Release of Fluconazole
1	0	0	0
2	15	3.184596	5.71750285
3	30	5.166919196	12.56385405
4	45	7.125378792	18.1559293
5	60	9.121338388	23.89338654
6	90	11.07979798	29.4541049
7	120	13.05075758	35.03762828
8	180	14.99671718	40.54703535
9	240	17.03017677	46.07354618
10	300	18.98863637	51.37725199

RESULTS AND DISCUSSION: Amphoteracin-B and fluconazole microemulsion was prepared using constituents such as oleic acid, propylene glycol, tween80 (non-ionic surfactant) and water. Non–ionic surfactant was more convenient to be used due to their less toxic and less skin irritation effects.

Table 10: Viscosity measurements

Viscosity measurements

Comparative Viscosity values of Formulations

Formulations	Viscosity(cps)*
ME-1	52.6±0.6
ME-2	75.3±0.8
ME-3	91.4±0.4
ME-4	103.5±0.5
ME-5	118.2±0.2

***Values are mean** ±SD, n=3

Mechanical stress study

The following table demonstrates the mechanical stress study of different formulations developed. The highest % phase separation was recorded as 10 after exploring 60 minutes centrifugation time. The minimum % phase separation was noted as 2 after 10 exploring 10 minutes centrifugation time.

Table 11: Mechanical stress study.

Mechanical stress study

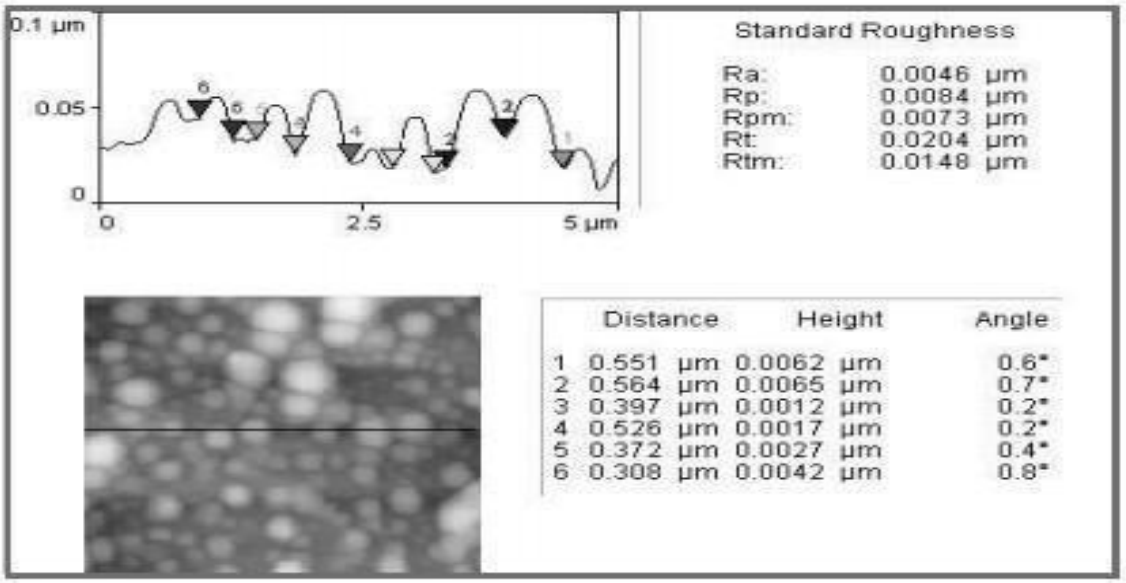
Comparative studty of mechanical stress in Formulations

S.No	Centrifugation time (min)	% Phase separation				
		ME-1	ME-2	ME-3	ME-4	ME-5
1	10	-	-	-	-	2
2	30	4	-	-	8	6
3	60	8	2	-	12	10

Particle size range

The below table demonstrates the particle size range of formulation. It depicts that particle size ranges in diverse sizes as per micro emulsion.

particle size range of Formulation (ME-3)



CONCLUSION : The Microemulsions of Amphoteracin-B and fluconazole were successfully formulated using various ratios of different types of excipients. From the present study it was concluded that microemulsion may have number of advantages like enhanced drug solubility, good thermodynamic stability, ease of production and enhanced effect on transdermal layer. Apart from that, amphoteracin-B and fluconazole microemulsion system could be the most useful and convenient topical formulation for the patient who are not able to take drug orally. This research comes under the New Drug Delivery System (NDDS) that enhances the new approach in frequent delivery of loaded Amphoteracin-B and fluconazole microemulsion.It would be a great launch towards allopathic medicines to ease the life and millions. It may also be confirmed that its production at bulk level would be reasonable in terms of cost. It will reduce the dosing frequency of the same microemulsion.

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