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PREPARATION AND EVALUATING PRONIOSOMAL GEL OF ISOCONAZOLE

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ABSTRACT
A fungus is a germ that ranges from something tiny and barely visible all the way to something big like a mushroom. They are all around us and don't usually cause any problems. Occasionally fungi (which is the plural of fungus) can cause more serious infections, particularly if the host is already ill. This can happen if the host is taking medicines that lower the immune system, like chemotherapy. A fungus is just a type of germ. They can range from tiny, barely visible specks that float around in the air, all the way up to large growths like mushrooms. Fungi (the plural of fungus) are all around us and don't usually cause any problems. An existence of transdermal delivery tool, proniosomal gel, has established to showed remarkable development for lipophilic/hydrophilic drugs over additional formulations. This research work has aimed to develop and evaluate proniosomal gel of isoconazole. The research aimed to evaluate the drug on the basis of its Physiochemical properties of isoconazole, Melting point, Solubility studies, Calibration curve using UV spectrometry, and Drug excipient compatibility study. The drug was studied at 40ºC ± 75% RH for 1 month and was also studied at room temperature for same time period.

Keywords: proniosomal gel, isoconazole, fungal infection, antifungal.

1. INTRODUCTION

Fungal infections can affect anyone, and they can appear on several parts of the body. A jock with athlete's foot, a baby with thrush, and a woman with a vaginal yeast infection are just a few examples. [1]

Fungi are microorganisms characterized by a substance in their cell walls called chitin. Some fungi, like many types of mushrooms, are edible. Other types of fungi, like *aspergillus*, can be extremely dangerous and lead to life-threatening diseases.

Different types of fungi can cause fungal infections. In some cases, fungi that aren't typically found on or inside your body can colonize it and cause an infection. In other cases, fungi that are normally present on or inside your body can multiply out of control and cause an infection.

Fungal infections can be contagious. They can spread from one person to another. In some cases, you can also catch disease-causing fungi from infected animals or contaminated soil or surfaces.

If you develop signs or symptoms of a fungal infection, make an appointment with your doctor.

Generally, IFIs are infections of immunocompromised hosts. The standard meaning of the immunocompromised host is growing from the customary arrangement of patients with AIDS, patients with tumor who are experiencing immunosuppressive chemotherapy or transplant patients whose resistant framework are smothered to forestall organ rejection. Additionally, IFIs are likewise seen in patients who are solid and obviously immunocompetent however who have hidden, asymptomatic condition that may adjust safe capacity and incline toward infections, for example, the nearness of against cytokines, for example, GM-CSF.

Anyway, these medications similarly as with any treatment with have constraints and provisos. For instance, toxicities related with the utilization of some antifungal specialists can be restrictive toward utilize or should be acknowledged keeping in mind the end goal to successfully treat the patient.

1.1. TYPES OF FUNGAL INFECTIONS

There are more than 100 different types of fungal infections with different types and causes and treatment methods three of the most common types. [2]

a. CUTANEOUS FUNGAL INFECTIONS

Superficial parasitic diseases (superficial mycoses) can be spread effectively however coordinate contact with tainted individuals, creatures, apparel, brushes and other object. The fungi tend to grow in moist part of the body where the skin comes together such as between fingers, toes, breast and in genital area. For instance, foot growth fungus (tinea pedis), ring worm, candidiasis.

b. SUB CUTANEOUS FUNGAL INFECTIONS

Affirmed by the exhibit of fungal grains in discharge or tissue biopsy culture is normally fundamental for species recognizable proof particular antibodies can as a rule be identified by precipitation for instance Actinomycetoma.

c. SYSTEMIC FUNGAL INFECTIONS

fungus in the blood and tissue (immunocompromised populace, generally life threating) powerless populace stomach medical procedure, cancer disease, chemotherapy, bone marrow transplant other immunecompromising disease, for example histoplasmosis, Aspergillosis.

1.2. Causes of fungal infections

Fungal infections are caused by hundreds of fungi that exist in our everyday environment. Most people can be exposed to fungi regularly without an adverse reaction, but certain conditions can cause the fungi to overgrow and cause symptoms. Those conditions include:

- Weakened immune system
- Travel to an environment with excessive fungi
- Outbreak of fungi due to changes in the environment, such as construction
- Introduction of new fungi to an environment

Diagnosing fungal infections

Diagnosis of a fungal infection will begin with a physical exam and discussion of your symptoms. For a fungal skin infection, your physician may take a scraping of your skin, a hair sample or a nail clipping for analysis at a lab to determine the type of fungus causing the infection.

For fungal infections affecting other parts of the body, your physician may take a sample of bodily fluids, including:

- Blood
- *Sputum* (the mucus in your respiratory system)
- Urine
- *Cerebrospinal* fluid (the fluid surrounding your brain and spinal cord)

In some cases, your physician may take a *biopsy* (tissue sample) of the affected organ. In the case of fungal masses in the respiratory system, an X-ray can help determine the amount of tissue damage.

2. MECHANISM OF ACTION OF ANTI FUNGAL DRUG

Cells of fungus are mind boggling living being that offer numerous biochemical focuses with their eukaryotic cells along these lines specialists that cooperate with contagious targets not found in eukaryotic cells are required the contagious cell divider is a one of a kind organelle that satisfies the criteria for particular poisonous quality. Albeit every life form has an alternate biochemical synthesis their gross cell divider structure is comparative. There is general three general component of activity for antifungal specialist cell membrane interruption, restraint of cell division and hindrance of cell divider arrangement.

I. INHIBITION OF CELL WALL FORMATION

Obstruction with parasitic cell divider biosynthesis has not been as fruitful and successful as penicillin and cephalosporin against bacteria numerous synthetic compounds have been found that meddle with different strides in contagious cell divider synthesis with phenomenal antifungal action in vitro. Shockingly improvement of these specialists into helpful medications has demonstrated extremely troublesome. A significant number of these operators are produced to target B-glucagon synthesis.

II. CELL MEMBRANE DISRUPTION

Antifungal specialists that disturb the cell layer do as such by focusing on ergosterol either by authoritative to the sterol, shaping pores and making the film wind up cracked (as with polyene antifungals), or restraining ergosterol biosynthesis (as observed with azole antifungal agents). Ergosterol is like mammalian cholesterol, along these lines operators restricting ergosterol may have a cytotoxic impact in the host tissue. Ergosterol has two conjugated twofold bonds that are deficient in mammalian sterols.

NEWER APPROACHES FOR ANTI FUNGAL DRUG ACTION

The possibility of parasitic particular targets has been exceedingly alluring, because of the low potential for off-target impacts in the human host. Such has been the situation with the cell divider repressing echinocandins. Late research has investigated the trehalose biosynthesis pathway as a parasitic particular process required for destructiveness. Past investigations have demonstrated that trehalose generation is required for the destructiveness of a few parasitic animal types. For instance, interruption of both Candida albicans TPS1 and TPS2, the qualities encoding trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase, separately, prompts diminished development at high temperatures, and additionally a hyphal arrangement imperfection. In Aspergillus fumigatus, the homolog to TPS2, the second quality in the trehalose biosynthetic. Pathway is required for virulence, however has no impact on trehalose biosynthesis. [14] Other specialists discovered that trehalose biosynthetic qualities are required for high temperature development and stress protection in both Cryptococcus neoformans and C. Gattii. Moreover, cancellation of the second quality engaged with trehalose biosynthesis, TPS2, prompted the accumulation of trehalose-6-phosphate and cell demise, proposing that blockade of biosynthesis now could give an intense effect to treatment because of middle of the road metabolite toxicity for the pathogen. The recent assurance of a crystal structure for C. albicans Tps2 takes into account further understanding and displaying of compound-catalyst interactions, taking into account the identification of potential inhibitors that specifically tie to this extraordinary focus for antifungal medication development. Additionally, another conserved protein being investigated as an antifungal medication target is the Hsp90 Heat shock protein. This Hsp90 molecular chaperone is associated with protein collapsing because of numerous cellular anxieties. Parasitic Hsp90 is

remarkably associated with resistance to antifungals, including both azoles and echinocandins, in Candida albicans, C. glabrata and Aspergillus fumigatus. Hsp90 inhibitors apply intense activity in combination with different antifungals. For instance, the Hsp90 inhibitor geldanamycin has a powerful fungicidal effect against azole-safe A. fumigatus when utilized as a part of combination with caspofungin or the calcineurin inhibitor FK506.

3. LITERATURE REVIEW

[3] Ketoconazole existing oral formulations suffer poor bioavailability since KTZ undergoes a marked first-pass effect and its absorption is dissolution rate-limited. In this study, a novel sustained release proniosomal system was designed using different non-ionic surfactants in which proniosomes were converted to niosomes upon skin water hydration following topical application under occlusive conditions. Different in vitro aspects (encapsulation efficiency, vesicle size and shape, in vitro release and stability) were studied leading to an optimized formula. All formulae exhibited high entrapment efficiencies, regardless of the surfactant HLB. Vesicle size analysis of promising formulation showed that all vesicles were in the quality range which favored efficient transdermal delivery. The entrapment efficiency of drug in optimized formulation (F3) containing Span 60 is high (94.93%) moreover, the extent of drug permeation through the membrane from the optimized formula was also quite high (93.52%) after 24 hrs. Concentration of cholesterol and lipid also plays an imp role in the entrapment efficiency of formed proniosomes. The above results indicate that the proniosomal gel of KTZ could be formulated for sustained release using optimum concentration of cholesterol, lipid and suitable surfactant to deliver a desired concentration of drug at site of action and overcoming the side effects of oral route.

[4] The present research has been undertaken with the aim to develop a topical gel formulation of Itraconazole. Itraconazole is an imidazole derivative and used for the treatment of local and systemic fungal infection. The oral use of Itraconazole is not much recommended as it has many side effects. Commercially Itraconazole topical gel preparation are not available in the market, thus this formulation is made for better patient compliance and to reduce the dose of the drug and to avoid the side effects like liver damage and kidney damage. The gel was formulated by changing the polymer ratio. Various formulation (F1, F2, F3, F4, F5) were developed by using a suitable polymer (Carbopol 934p and HPMC). The formulation was evaluated for % yield, spreadability, extrudability, wash ability and viscosity in vitro drug release study, skin irritation study, stability testing. Viscosity studies of various formulations revealed that formulation F3 was better to compare to others. From among all the developed formulation, F3 shows better drug diffusion, did good Rheological properties. pH of the F3 formulation is sufficient enough to treat the skin infections. Results indicated that the concentration of carbopol-934 and HPMC K4M significantly affects drug release and rheological properties of the gels.

[5] Miconazole nitrate is a broad-spectrum antifungal agent, and it is used for the treatment of superficial fungal infections. It has low skin permeability. Therefore, the aim of this study was to prepare proniosomal 2% miconazole nitrate sustained release to a treat deep- seated fungal infections. The different batches of proniosomal 2% miconazole nitrate gel were prepared by the conservation phase separation method using different non-ionic surfactants and Cholesterol. Preliminary trial batches were formulated and evaluated for different evaluation parameters like pH, viscosity, % entrapment efficiency, % drug content, and in-vitro drug release study. A 32 full factorial design was used to check the effect of Span 60 (X1) and Cholesterol (X1) on %

entrapment efficiency (EE) and % drug release at 20 h (Q20). Multiple linear regression analysis, ANOVA, and graphical representation of the influence factor by 3D response surface plots were performed using Design Expert 9. Checkpoint batch was prepared to validate the evolved model. Optimized batch was found to be stable, and it showed release 94.76% in 24 h. It followed the non-fickian diffusion and showed flux 289 µg/cm2/h in the ex-vivo study. SEM revealed that the noisome formed were spherical in shape. Therefore, proniosomal 2% miconazole nitrate gel has the ability to penetrate the skin and give the effect for a long time.

[6] Fungal skin infections are the most common global issue for skin health. Fungal infections are often treated by topical or systemic anti-fungal therapy. Topical fungal therapy is usually preferred because of their targeted therapy and fewer side effects. Advanced topical carriers because of their distinct structural and functional features, overcome biopharmaceutical challenges associated with conventional drug delivery systems like poor retention and low bioavailability. Literature evidence indicated topical nanocarriers loaded with anti-fungal agents display superior therapeutic response with minimum toxicity. Nanocarriers often used for topical anti- fungal medication includes Solid-Lipid nanoparticles, Microemulsions, Liposomes, Niosomes, Microsponge, Nanogel, Nanoemulsion, Micelles etc. This review summarizes recent advances in novel strategies employed in topical carriers to improve the therapeutic performance of anti-fungal drugs

[7] Azole derivative- based antifungal creams, liquids, or sprays are available to treat fungal infections; however, these formulations show various side effects on the application site. Over the past few years, herbal extracts and various essential oils have shown effective antifungal activity. Additionally, autoxidation and epimerization are significant problems with the direct use of herbal extracts. Hence, to overcome these obstacles, polysaccharide-based nanohydrogels embedded with natural plant extracts and oils have become the primary choice of pharmaceutical scientists. These gels protect plant-based bioactive compounds and are effective delivery agents because they release multiple bioactive compounds in the targeted area. Nanohydrogels can be applied to infected areas, and due to their contagious nature and penetration power, they get directly absorbed through the skin, quickly reaching the skin's third layer and effectively reducing the fungal infection. In this review, we explain various skin fungal infections, possible treatments, and the effective utilization of plant extract and oil-embedded polysaccharide-based nanohydrogels.

[8] Global incidence of superficial fungal infections caused by dermatophytes is high and affects around 40 million people. It is the fourth most common cause of infection. Clotrimazole, a broad-spectrum imidazole antifungal agent is widely used to treat fungal infections. Conventional topical formulations of clotrimazole are intended to treat infections by effective penetration of drugs into the stratum corneum. However, drawbacks such as poor dermal bioavailability, poor penetration, and variable drug levels limit the efficiency. The present study aims to load clotrimazole into ufosome and evaluate its topical bioavailability. Clotrimazole loaded ufosomes were prepared using cholesterol and sodium oleate by thin film hydration technique and evaluated for size, polydispersity index, and entrapment efficiency to obtain optimized formulation. Optimized formulation was characterized using scanning electron microscopy (SEM), X-ray diffraction (XRD), and differential scanning calorimetry (DSC). Skin diffusion studies and tape-stripping were performed using human skin to determine the amount of clotrimazole accumulated in different layers of the skin.

4. RESEARCH METHODOLOGY

The research methodology involved finding of the following:

Pre-formulation things about the study

- Physiochemical properties of isoconazole.
- Melting point.
- Solubility studies.
- Calibration curve using UV spectrometry
- Drug excipient compatibility study

Formulation studies:

- Selection of optimize batch on the basis of three-square factorial design.

Determination of melting point (M.P)

Melting point of the drug was determined with digital melting point apparatus (VEEGO-VMP-D) The capillary was sealed from one end by holding it on the flame; from the other end drug was filled into the capillary up to 1cm and was kept in the capillary holder of the instrument. Heat was supplied at the rate of 5ºC/min and the drug was observed for melting.

Determination of calibration curve

Calibration curve of Isoconazole in Ethanol

10 mg of the drug was dissolved in 10 ml of ethanol.1ml of resulting solution was diluted to 10 ml with ethanol. This 10 ml solution (100µg/ml) was used as stock solution for the preparation of further dilutions. Suitable dilutions were made with water to get solutions of 10-23 µg/ml and absorbance measured at 263 nm. Plot of absorbance vs. concentration was plotted and analyzed regression coefficient.

Calibration curve of Isoconazole in Phosphate Buffer (pH 7.4)

10 mg of the drug was dissolved in 10 ml of Phosphate Buffer (pH 7.4) .1 ml of resulting solution was diluted to 10 ml with Phosphate Buffer. This 10 ml solution (100µg/ml) was used as stock solution for the preparation of further dilutions. Suitable dilutions were made with water to get solutions of 10-20 µg/ml and absorbance measured at 263 nm. Plot of absorbance vs. concentration was plotted and analyzed regression coefficient.

Compatibility between drug and excipients FTIR spectra analysis

FTIR spectroscopy is one of the qualitative analytical techniques, which offer the possibility of detecting chemical interaction. FTIR analysis Spectra of Isoconazole and excipient was determined on Fourier Transform Infrared spectrometer. The FTIR was performed on Isoconazole, span 60, cholesterol and physical mixture of Isoconazole and excipients to know the possible chemical interaction.

FORMULATION STUDY

Preparation method of Isoconazole proniosomal gel.

Proniosomal gel was set up by a coacervation-stage division strategy. Exactly measured measures of surfactant, cholesterol and medication were taken in a spotless and dry wide mouthed glass vial of 5.0 ml limit and liquor (0.5 ml) was added to it. Every one of the fixings were blended well with a glass bar and the open end of the glass bottle was secured with an aluminium thwart to keep the loss of dissolvable from it and detailing was warmed over water shower at 65°C ± 5ºC for around 5 min until the point when the surfactant blend was broken down totally. At that point 1 ml of phosphate support (pH 7.4) was added to the definition and was warmed on water shower until the point when clear arrangement was acquired. The blend was permit to cool at room

temperature until the point when the blend is changed over to proniosomal gel. The proniosomal gel was mixed with 2% of polymeric gel (HPMC k-15) to produce desired viscosity.

Experimental design

A three square experimental deign consisting 3 level and 2 factors was applied to optimize the formulation

Development. It was suitable for investigating the quadratic response surfaces using Design Expert 10.0 (Version) software. In this study, independent variables (i.e., concentration of surfactant and cholesterol) there low level, medium level and high-level concentration was determined and according to the design the batches were taken which significantly influence the observed response for particle size, entrapment efficiency (dependent variable).

5. RESULT AND DISCUSSION

PREFORMULATION STUDY

Solubility studies

Isoconazole was found to be partially insoluble in the water. It was freely soluble in ethanol and phosphate buffer (pH 7.4).

Determination of the Melting point

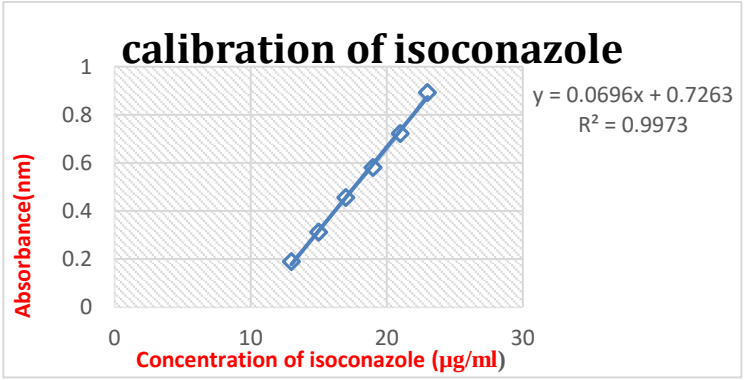
The melting point of Isoconazole was found to be 175ºC which is to be accordance with the reported value which is 179ºC.

Calibration curve

Calibration Curve of isoconazole in ethanol

Calibration curve was found to be linear over the concentration range of 13-23µg/ml at 263 nm by UV- spectrometry, with the equation of line as y = 0.0696x + 0.7263 respectively and R^2 = 0. 9973.plot of Absorbance vs. concentration was plotted by using Microsoft excel.

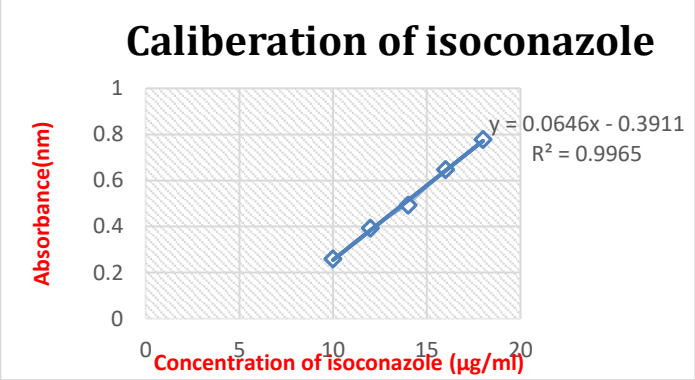
calibration of isoconazole in ethanol.



Calibration Curve of isoconazole in Phosphate Buffer (pH 7.4)

Calibration curve was found to be linear over the concentration range of 10-23µg/ml at 263 nm by UV- spectrometry, with the equation of line as y = 0.0696x + 0.7263 respectively and R^2 = 0.9973. Plot of Absorbance vs. concentration was plotted by using Microsoft excel.

Calibration of isoconazole in Phosphate Buffer (pH 7.4)



COMPATIBILITY BETWEEN DRUG AND EXCIPIENTS FTIR SPECTRA ANALYSIS

Fourier transform infrared spectroscopy results confirmed the physical as well as chemical stability and compatibility of isoconazole and excipients. The figure exhibited the spectra of a) isoconazole b) physical mixture of drug and excipient. Native Isoconazole showed the characteristics band due to different functional groups which are clearly shown in the physical mixture of the drug and excipient which ensures that there is no chemical interaction between drug and excipients.

FORMULATION STUDY

Fabrication study of the Isoconazole transdermal gel

The preparation of the transdermal gel in this work utilized simple, one step and quick process of incorporating the drug into the vesicles and thus formation proniosomal gel.

Surfactant and cholesterol used both play important role in the formation of the proniosomes. HLB value indicates the formation of vesicle or not. Span 60 surfactant have the HLB value of 4.7 which is considered to be high range in the formation of the vesicle. Span 60 reduces the surface free energy which produced the large vesicles formation which gives the large exposure to the area of skin and dissolution medium. Transition temperature of surfactants affects the entrapment of drug in vesicles. Spans 60 with highest phase transition temperature provide the highest entrapment for the drug. Traverse 60 produces vesicles of bigger size with higher entanglement of medication. The medication draining from the vesicles is decreased because of high stage change temperature and low penetrability.

Cholesterol is fundamental part of vesicles development. Consolidation of cholesterol impact vesicle security and penetrability. Grouping of cholesterol assumes an essential part in ensnarement of medication in vesicles. The ensnarement proficiency increments with expanding cholesterol content and by the utilization of traverse 60 which has higher progress temperature. High cholesterol content loweringly affected medication entanglement to the vesicles this could be because of the way that cholesterol past a specific level begins upsetting the customary bi-layered structure prompting loss of medication entrapment.so as much as cholesterol content was kept low in detailing.

Ethanol utilized as the dissolvable in detailing, it has the impact on vesicle development. Ethanol gives the most elevated size because of the high fluid solvency. Phosphate support is utilized to keep up the pH of the detailing close to the human skin and also choice of the pH has impact in the capture of the medication.

Mechanism of action of proniosomes.

EVALUATION OF THE ISOCONAZOLE PRONIOSOMAL GEL

Physical appearance

The proniosomal gel was observed visually and found to be clear and free from air bubbles.it was observed under microscopes to see any particles are present or not and found free from particles. The proniosomal gel was easily spreadable and provide smooth texture.

Viscosity

The proniosomal gel viscosity was found to be good. The gel remains adhere to the human skin for long period of time. The viscosity was measured by the Brookfield viscometer and found to 4855±0.13.

pH measurement

The pH of the transdermal gel was found to be in range of 5.0-6.5 which is similar to the skin pH, this ensures that the there is no irritation of the gel on the skin and hence more acceptance by the patients due to less irritation to skin.

Drug entrapment efficiency

Vesicular entrapment efficiency is an important parameter that conveys the stability of vesicles and this depends upon the amount of surfactant as well as amount of cholesterol used. The entrapment efficiency of these formulation varies from 58.11 to 76.82%. Effect of independent variables on the entrapment efficiency can be represented in the 3D surface plot using Design Expert 10.0® (Version) software.

Drug entrapment efficiency

Formulation Batch	% Drug Entrapment
PNI	63.23
PN2	31.07
PN3	64.72
PN4	76.82
PN5	73.91
PN6	69.57
PN7	65.02
PN8	61.88
PN9	58.11

6. CONCLUSION

The melting point of Isoconazole was found to be 92ºC which is to be accordance with the reported value which is 94ºC. Isoconazole was found to be partially insoluble in the water. It was freely soluble in ethanol and phosphate buffer (pH 7.4). Calibration curve was found to be linear over the concentration range of both 13-23µg/ml at 263 nm and 10-23µg/ml at 263 nm by UV-spectrometry. Native Isoconazole showed the characteristics band due to different functional groups which are clearly shown in the physical mixture of the drug and excipient which ensures that there is no chemical interaction between drug and excipients. The entrapment efficiency of these formulation varies from 58.11 to 76.82%. From these results, it can be concluded that the drug developed through this research is effective in treating fungal infections.

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