ISSN No:-2456-2165

# Development and Evaluation of a Novel Transungual Formulation (Nail Patch) for the Treatment of Onychomycosis

Samyuktha Metta[1], Dr. K.S .Murali Krishna[2,] Swapna. Kandukuri[3],
Sravya Maddukuri[4] , Swarna Latha Nagadhani[5]
[1]Assistant Professor, Department of Pharmaceutics, M L R Institute of Pharmacy, Hyderabad,Telangana,India
[2] Principal, Professor, Department of Pharmacognosy, M L R Institute of Pharmacy, Hyderabad,Telangana,India
[3, 4, 5] Assistant Professor ,Department of Pharmaceutics, M L R Institute of Pharmacy, Hyderabad, Telangana, India

ISSN No:-2456-2165


## ABSTRACT

**Nail may be defined as translucent plates of hard keratin lying fused with nail bed except at distal end. Toe nails and finger nails protects the tissues of toes and fingers. Nail are made up of a harden protein known as Keratin, which are also present in hair and skin. The health of nails can be a better clue of overall health. Nail diseases are mainly associated with conditions like bacterial & fungal infections, iron deficiency anemia, congenital defects. Toenail fungal diseases are much more common than finger nail fungal diseases. Onychomycosis or tinea unguium is most common toenail fungal infection usually caused by a special type of fungus known as a dermatophyte.this toenail fungal infection is responsible for approxiamatelt 50% of all nail disorders characterized by discoloration and thickening of the nail and which also effects nail plate,nail bed and nail flods(1).Since most of these infections are relatively superficial,topical therapies are commonly used for treatment which are limited by keratinized cells in human nail plate. This type of topical therapeutic application is not sufficient to provide a successful therapy of such nail diseases as the nail unit is relatively impermeable. Hence current research work focus on nail permeation by altering the nail plate barrier by means of permeation enhancers.A novel transungual formulation(Nail patch ) is desirable in treartment of nail diseases like Onychomycosis.Research work main aim is to formulate and evaluate matrix –type transungual delivery system containing an antifungal drug,Itraconazole with different permeation enhancers (in 2 different ratios) by the film casting technique and explores the effect of permeation enhancers on the invitro permeability of Itraconazole across nail.Matrix transungual patches were prepared by using hydroxyl propyl methyl cellulose (HPMC E 5) and ethyl cellulose (EC) as polymers,Eudragit RL 100 as pressure sensitive adhesive by incorporating di ethyl phthalate as plasticizer and Propylene Glycol, Poly ethylene glycol, and oleic acid as permeation enhancers in 2 different ratios of 4% and 6% v/v in whole formula.Prepared patches were subjected to different evalution studies in which permeation studies were performed by using Franz diffusion cell apparatus. All the formulated patches that evaluated shows uniformity with respect to physiochemical evaluation. The in-vitro permeation study indicated that formulation INP-4 propylene glycol (6%) showed maximum release of 74.719 % in 12 hrs emerging to be ideal formulation. The developed transungual patches shows increase in the efficacy of Itraconazole for the treatment of a fungal disease such as Onychomycosis.**


***Keywords:-*** *Onychomycosis , Transungual, Permeation enhancers, Itraconazole, in-vitro permeation study .*

ISSN No:-2456-2165

## I. INTRODUCTION

Over the years, the human nail has become a fashion accessory, and even with a slacking economy, people are willing to spend money for inexpensive ways to make their nails look good. Continuous oral medication may affect all organs, often causing kidney damage and anti-fungal class Itraconazole drug may produce nausea/vomiting, diarrhoea, gas, headache, dizziness or stomach upset[(2)]. Some infections,trauma, diseases,improper growth and use of some medications may responsible for the cause of nail disorders.

Certain infections of the nail are caused by bacteria,fungi and virus. Common diseases of nail may include Onychomycosis, Onychatrophia, Onychogryposis, Leuconychia, Koilonychia, Onychauxis,psoriasis,onychocryptosis,onychorrhexis,psoriatic onychodystrophy etc., There are variety of topical formulations like gels,creams and also ointiments which are commonly used for the treatment of nail infections but affect in the treatment is limited because of their relatively low impermeability. Present work involves the formulation of transungual drug delivery which is a new drug delivery system to treat nail infection with effective permeation. Transungual topical administration of therapeutic agents offers many advantages over conventional oral and invasive methods of drug delivery, and also provides controlled release of the drug for extended period of the time [(3)]. Topical delivery of antifungal agents through the human nail offer several advantages over oral therapy including lower incidence of adverse events and lower potential for drug-drug interaction with drugs used to treat diabetes, HIV/AIDS and psoriasis. Transungual drug delivery system has been accepted as potential non-invasive route of drug administration, with advantages of prolonged therapeutic effect, reduced side effects, improved bioavailability, decreased hepatic metabolism, predetermined rate of drug permeation, better patient compliance and easy termination of drug therapy[(4)].

Transungual is a greek word that is derived from two words “Trans” means “Through” and “Unguis” means “Nails”.Transungual drug delivery system is a system associated with drug delivery through the nail to achieve a target drug delivery system of the nail to treat the nail diseases . Nail plate is main route for penetration of drug across it and delivery of the drug through nail (nail plate) is known as transungual drug delivery system[(5)].

ISSN No:-2456-2165

## II. ONYCHOMYCOSIS

Onchomycosis (Tinea unguium) is a fungal toe nail infection which is responsible for about 50% of other nail disorders. Approximately 5% of worldwide population affected by Onychomycosis.[6,7].the word Onchomycosis is derived from two greek words ,namely onyx-anail,mykes-a fungus.It may affects any part of the nail unit such as nail plate,nail bed and nail matrix[8].

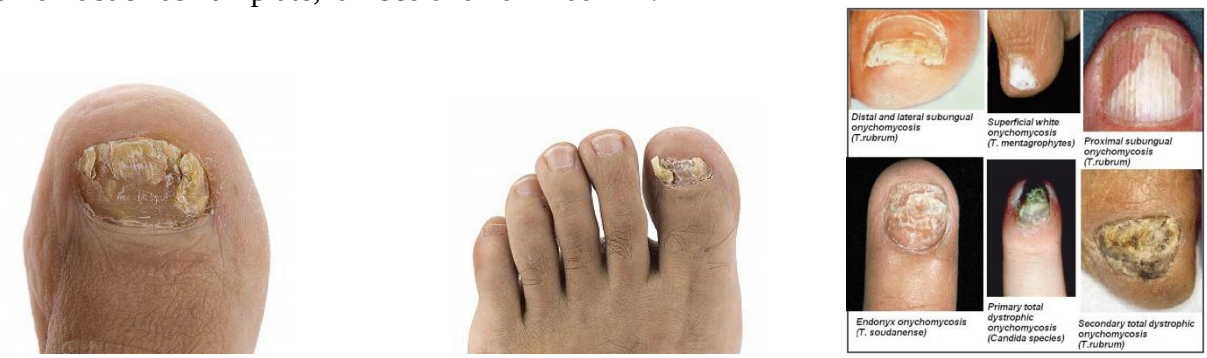


Fig 1:- Onychomycosis

Onychomycosis is a common, chronic and hard to eradicate fungal disease of toe nails and finger nails affecting 10-30% of the population globally. Clinically onychomycosis presents with discoloration, thickening and irregular surface. It is responsible for approximately 50% of all nail disorders. Risk factors for nail infection are diabetes, age, smoking, compromised immune system such as in HIV and peripheral vascular diseases [9].Transungual drug delivery systems with antifugal agent are novel preparations used to treat diseases such as onychomycosis. Use of this system avoids oral toxicity of anti fungal drugs [10].

### *A. Tissues affected by Onychomycosis.*

Onychomycosis (Tinea unguium) is a fungal nail infection which mainly affects human toe nails.The nail is a highly differentiated appendage of the skin serving protective functions.[11] Apart from their protective functions, the nails also play a role in amplifying the sense of touch in the fingertips [12].The nail apparatus consists of the nail plate ,nail bed ,nail folds,nail matrix and the hyponychium,[13] as shown in figure 2 .The human nail is composed on 80-90 layers of dead ,highly keratinized flattened tightly bound cells which lack cell organelles and nucleus . The nail plate is transculent, but appears pink is color due to the rich vasculature in the nail bed underneath . The nail matrix is thick, specialized germinative epithelial structure responsible for generation of bulk of the cornified cells of the nail plate [14]. The process of keratinization is different in the nail matrix relative to that in the epidermis because; the onychocytes in the nail matrix undergo complete keratinization without formation of keratohyalin granules. The proximal nail fold covers and protects the nail matrix.The newly formed nail emerges out of the proximal nail fold and is supported by the nail bed. The nail bed extends from the lunula to the hyponychium .About 20% of the thickness and the mass of the ventral layer of the nail plate is produced by the nail bed [14]. The nail bed is viable epidermis. The inter digitations on the ventral side of the nail plate hold the nail plate firmly in the place with the nail bed .The lateral nail folds help to hold the nail plate in the place. The hyponychium is epidermis between the distal nail groove and the nail bed.The nail bed,the nail matrix and the nail folds are well perfused with blood and lymphatic vessels [15, 16].

*B. Treatment available for Onychomycosis*[17]

Identified methods of treatment fall into three categories:

- Removal of all or division of the affected nails
- Oral/systemic therapy
- Topical/Ungual therapy

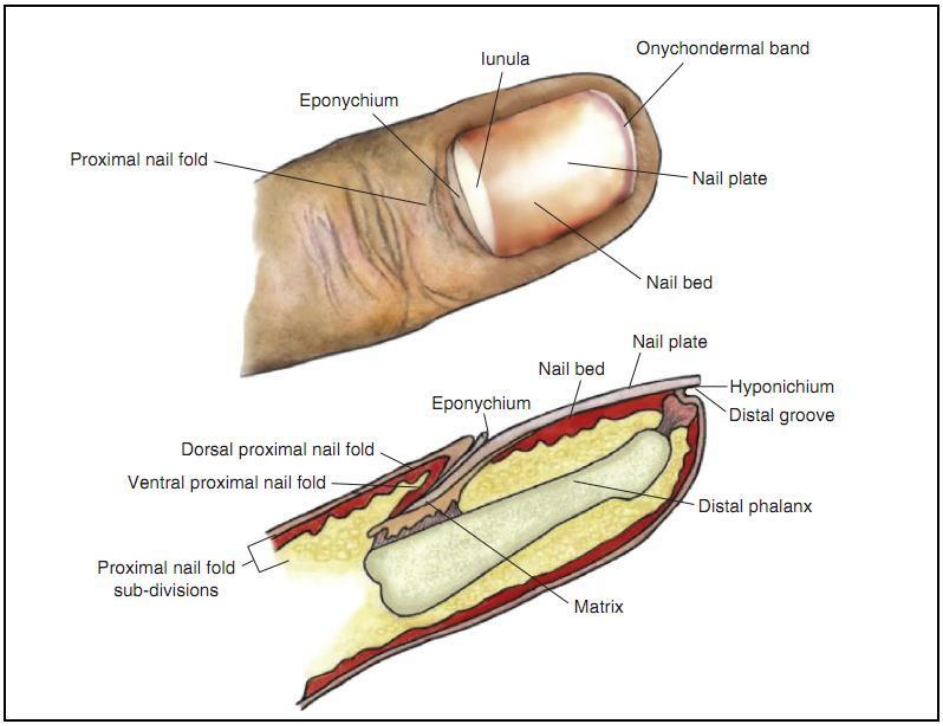


Fig 2:- Components of the Human Nail

ISSN No:-2456-2165

## III. MATERIALS AND METHODS

### *A. Materials*

Itraconazole was obtained as gift sample from Hetero Pharma LTD., Hyderabad. Polymers such as Hydroxy propyl methyl cellulose E5 (HPMC) ,Ethyl cellulose (EC) and Penetration enhancers such as propylene glycol, Poly ethylene glycol 400, Oleic acid was provided by the institute and Pressure sensitive Adhesive (Eudragit RL-100) was provided by Nacto Pharma Ltd. Analytiacal grade of chemicals such as Diethyl phthalate, Sodium hydroxide, Potassium dihydrogen Orthophosphate, Di chloro methane and methanol used and methanol were used for the study.

ISSN No:-2456-2165

## IV. METHODS OF PREPARATION

*A. Preparation of standard solution*

Accurately weigh 100mg of pure drug Itraconazole in 100ml volumetric flasks and dissolve in small quantity of 7.4 pH phosphate buffer inorder to form a clear solution.Then the volume was made with buffer solution upto 100ml mark so as to obtain a solution containing 1mg/ml[18].

*B. Preparation of stock solution*

From the standard solution,a stock solution was prepared by pipette out 1ml of above standard solution in a 100ml volumetric flask and then the volume was with 7.4 pH phosphate buffer solution,to give a solution containing 100 µg/ml.

*C. Preparation of working standard solution*

Series of aliquoters 2,4,6,8,and 10 ml of stock solution were pipette out into different volumetric flasks of 10ml volume.The volume was made upto the mark with 7.4 pH phosphate buffer.These dilutions indicates different concentrations namely 2,4,6,8 and 10 µg/ml concentration of Itraconazole.The prepared solutions were subjected for the measurement of absorbance at 262 nm by using UV spectrophotometer (Model---Shimadzu-1800) against an appropriate blank (7.4 pH buffer).

D. *Design and Formulation Development of the Transungual Patch*

The initial design for the transungual patch was based on the simple DIA ( Drug-in-adhesive) design[19] In order to meet the key properties for a successful topical patch a DIA type nail patch was designed as shown in figure 3 .

The nail patches were prepared by film casting technique using liquid Paraffin as lubricant. Prepared patches composed of four Layers.

- Backing membrane
- Pressure sensitive adhesive membrane (Eudragit RL100 5 % w/v of aqueous solution)
- Drug loaded HPMC film ( Itraconazole+PE+HPMC+EC+Plasticizer)
- Release linear (Peeling paper)

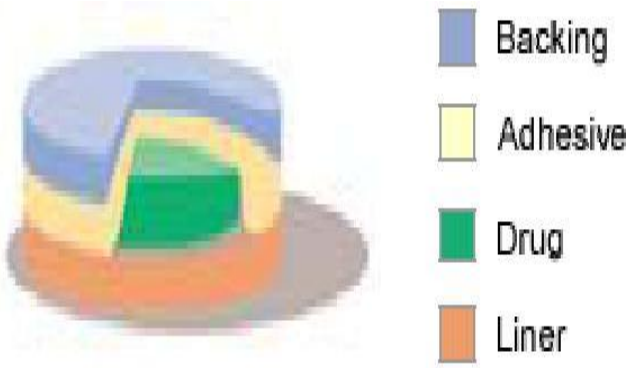


Fig 3:- Drug-matrix-in-adhesive

ISSN No:-2456-2165

| Formulation Code | INP1 | INP2 | INP3 | INP4 | INP5 | INP6 |
|---|---|---|---|---|---|---|
| Itraconazole (mg) | 200 | 200 | 200 | 200 | 200 | 200 |
| HPMC(mg) | 800 | 800 | 800 | 800 | 800 | 800 |
| Ethyl cellulose(mg) | 200 | 200 | 200 | 200 | 200 | 200 |
| Eudragit RL 100 (%W/V) | 5 | 5 | 5 | 5 | 5 | 5 |
| OleicAcid (%) | 4 | 6 | --- | --- | --- | --- |
| Propylene Glycol (%) | --- | --- | 4 | 6 | --- | --- |
| PEG 400 (%) | --- | --- | --- | --- | 4 | 6 |
| Diethyl phthalate (ml) | 5 | 5 | 5 | 5 | 5 | 5 |
| DCM: Methanol(ml) | 15 | 15 | 15 | 15 | 15 | 15 |

Table 1. Formulation table of transungual patches of Itraconazole

*Each patch contains 200 mg of drug in 15.896 sq.cm area.
*Each Sq.cm contains 12.5 mg of Itraconazole.
*INP=Itraconazole Nail patch.

The quantities of the components of the matrix were calculated as percentage (%) of dry weight polymer.

ISSN No:-2456-2165

## V. FABRICATION OF TRANSUNGUAL PATCHES

**Pressure sensitive adhesive membrane was prepared by using 5%w/v aqueous solution was poured onto the petri dish and followed by drying at $50^0$c for 8 hours.**

**Drug loaded HPMC film was prepared by dissolving require amount of HPMC and EC with equal amount of DCM and methanol and kept it for overnight for swelling .**

**The polymer solution was mixed with drug (Itraconazole),permeation enhancers and plasticizers onto the magnetic stirrer until a uniform solution was obtained .**

**Now uniform HPMC dispersion that obtain was casted on Pressure sensitive adhesive(Eudragit) membrane in a petridish ,which was then dried at room temperature for 2 hours by covering petri plates with funnels to avoid blistering effect after drying of patches .**

**Dry patches were removed and wrapped in aluminium foil and kept in dessicator for futher use**

**Prepared patches were stick to adhesive layer of bandage which can purchased from local market .**

Fig 4:- Prepared and Fabricated Itraconazole Transungual Patches

ISSN No:-2456-2165

## VI. EVALUATION OF TRANSUNGUAL PATCHES

Formulation development of a transungual dosage form involves a complex extensive research process.Transungual patches have been developed to improve the clinical efficacy of the therapeutic drug and there by enhancing the patient compliance by delivering smaller amounts of drug at predetermined rate.This ensures the importance of evaluation studies to define the patches performance and reproducibility .

*A. Physical appearance:*

All the formulated transungual patches were visually inspected for color,transparency,clarity,flexibility and smoothness.

*B. Patch Weight Uniformity:*

Weight variation is studied by calculating the average weight of randomly selected individually weighed patches.The individual weight that evaluated from weight variation test should not be deviated significantly form average weight.

*C. Patch Thickness uniformity:*

Patch thickness uniformity test was performed by measuring the formulated patch at 3 different points by using venier calipers and average of three readings was calculated.

*D. Folding Endurance:*

The folding endurance test of formulated patches was performed manually by folding the patch repeatedly at the same place (single point) till it broken.The number of times that the patch subjected to repeated folding at the same place without cracking/breaking indicates the folding endurance value . Folding endurance evauation test involves the determination of folding capacity of the films that subjected to folding at the frequent extreme conditions which also a indicative of brittleness.

*E. Percentage moisture absorption:*

Accurately weighed patches were placed in a desiccators containing 100ml of saturated solution of potassium chloride ,which maintains at 80-90% RH.After 3 days,the desiccated patches were taken out and subjected to weighing.

The percentage moisture absorption was calculated using the formula:

$$\text{Percentage moisture absorption} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100$$

*F. Percentage Moisture Loss:*

Accurately weighed patches were placed in a desiccator containing anhydrous calcium chloride. After 3 days ,the desiccated patches were taken out and subjected to weighing.
The moisture loss was calculated using the formula:

$$\text{Percentage moisture absorption} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100$$

ISSN No:-2456-2165

*G. Drug content uniformity*

Weigh accurately portion of patch (equivalent to 100 mg of drug) and dissolve in 100ml of phosphate buffer solution (7.4 pH ) in a 100ml volumetric flask .Place the flask on to the shaker for 24 hrs to achieve the complete dissolution.Then the obtained solution was filtered and the content was estimated spectrophotometrically at 262nm by appropriate dilution.

*H. Invitro Transungual permeation studies*

Franz diffusion cell or Keshary-chien diffusion cell was used to determine the invitro nail permeation of Itraconazole from various formulated transungual patches.Difussion cell composed of two compartments i.e.,donar and receptor.The receptor compartment comprises effective surface area of 3x3 $cm^2$ and 5ml capacity of volume.Permeation studies was carried by placing the fabricated patch with prehydrated nail in between receptor and donar compartment of the diffusion cell in which the receptor compartment was filled with 7.4 pH phosphate buffer .Transungual patch was placed in such a way that the nail facing towards the donor compartment and the patch towards the receptor compartment containing buffer solution[20] in which the receiver compartment was maintained at body temperature and was subjected to continuous stirring with the help of magnetic stirrer.

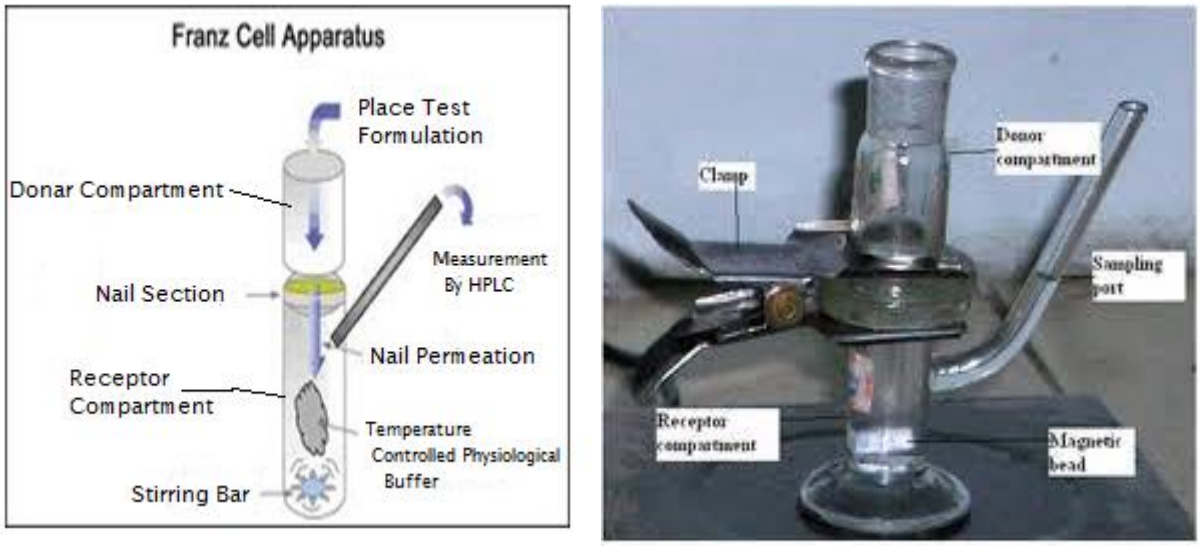


Fig 5:- Franz diffusion cell

Ideally dissolution medium of pH ranging between 5 to 8 and temperature that typically set at 37 $^{o}$C should be used which will reflecting skin and nail physiological conditions.100 rpm is a typical agitation rate for testing a patch section ( According to European Pharmacopoeia).A constant speed of agitation and temperature should be considered.

At predetermined time intervals the samples to be analysed are withdrawn and equal volume of pre-thremostated of fresh receptor fluid is replaced each time inorder to maintain skin conditions.Then the samples were analysed spectrophotometrically after appropriate dilution.

Type of design used to formulate transungual system,size of patch,thickness of nail etc., are some important variables that may affect the release of the drug from the system.

ISSN No:-2456-2165

## VII. RESULT AND DISCUSSION

- *Physicochemical evaluation*

*A. Physical appearance*

The Nail patches of formulations that prepared by using penetration enhancers such as oleic acid and propylene glycol are thin, transparent, flexible, smooth and uniform where as formulations prepared by PEG 400 yielded thick patches. The transparent nature of patches can be seen more prominently in formulations prepared by oleic acid. The flexibility can be due to HPMC. (Table -2).

*B. Patch weight uniformity*

The weights of all transungual patches were found to be uniform. The weight of formulations was determined by digital electronic balance and results are given in table-3. The weights of formulations were found to be in the range of 0.295gm. to 0.299 gm. This indicates that there is no significant weight variation in all formulations.

*C. Thickness uniformity*

Thicknesses of formulations were measured using venier calipers, and results are given in table-3.

Thickness of transungual patches were found to be in the range of 0.18mm to 0.25mm.The results showed that thickness was uniform for all the prepared transungual patches.

*D. Drug content uniformity*

Drug content uniformity of transungual patch was one of the important criteria which will ensure the uniform and reproducible sustained release of drug drug from the patch.The drug content uniformity of all the formulations was determined and the results indicated that the drug is uniformly distributed throughout the patches.the results of drug content uniformity in all the formulations were found to be in the range of 97.21% to 98.95%. (Table-3).

*E. Moisture Gain*

Among the formulations, INP5 showed maximum moisture uptake i.e. 2.93 % and INP4 showed minimum moisture uptake i.e. 2.19 %. (Table.3).

*F. Moisture Loss*

Among all the formulations,INP5 showed maximum moisture loss i.e. 1.56 % and INP2 showed minimum moisture loss i.e.1.10 %. (Table.3).

*G. Folding Endurance*

The ability of patch to withstand to rupture can be assessed by folding endurance measurement. All the patches were evaluated for folding endurance by folding the patch at the same place till it breaks. The folding endurance measurement shows that the patches were quite flexible and results was found to be satisfactory (Table-3).The results indicates that the patches would maintain integrity without any breaking upon general folding when used.The folding endurance values were found in the range of 250 to 316 times. The formulation INP2 was found to have lowest folding endurance, whereas formulation INP5 was found to have highest folding endurance. The folding endurance of patches increases with increase in the concentration of penetration enhancers.

ISSN No:-2456-2165

*H. In-vitro drug permeation*

The drug permeability depends on the penetration enhancer concentration and the cross link density of patches.Chemical properties of the drug,type of design as well as physicochemical properties of the dialysis membrane are the considerable characterstics which may influence the release of the drug from the transungual patch.

In the present study patches were prepared with different permeation enhancers in different concentrations.Penetration enhancers were used inorder to overcome the barrier properties of nail and skin. To enhance the permeation efficiency of drug ,chemical enhancers were used.

The in-vitro diffusion study was performed and data obtained from different formulations of Itraconazole transungual patches are shown in table 4.

The release of the drug from its transungual patch formulations can be ranked in the following descending order.

INP4>INP2>INP3>INP6>INP1>INP5

Where the amounts of the drug release of formulations INP1, INP2, INP3, INP4, INP5 and INP6 after 12 hours were found to be 64.916%, 72.065%, 70.186%, 74.719%, 64.916%, 67.131% respectively. INP4 shows highest cumulative amount of drug permeation at the end of 12 hours.

The highest % release from INP4 formulation may be due to the presence of high concentration of permeation enhancer propylene glycol. The permeability of Itraconazole with propylene glycol (6%) was found highest in our study.

| Formulation Code | Physical Appearance |
|---|---|
| F1 | Thin ,Transparent, flexible, Smooth |
| F2 | Thin, Transparent, Smooth, uniform, soft |
| F3 | Thin, Transparent Smooth, uniform |
| F4 | Thin, Transparent, Smooth, uniform, flexible |
| F5 | Smooth, uniform, Thick |
| F6 | Smooth , Sticky ,Thick |

Table 2. Physical appearance of Transungual Patches

| Formulation code | Weight uniformity(mg) | Thickness (mm) | Drug content uniformity(%) | Percentage Moisture gain (%) | Percentage Moisture Loss (%) | Folding Endurance |
|---|---|---|---|---|---|---|
| INP1 | 295 | 0.19 | 97.91 | 2.62 | 1.20 | 309 |
| INP2 | 299 | 0.18 | 97.86 | 2.82 | 1.10 | 316 |
| INP3 | 297 | 0.18 | 97.21 | 2.26 | 1.46 | 293 |
| INP4 | 298 | 0.20 | 98.95 | 2.19 | 1.30 | 297 |
| INP5 | 297 | 0.23 | 97.42 | 2.93 | 1.56 | 250 |
| INP6 | 299 | 0.25 | 98.85 | 2.23 | 1.36 | 257 |

*INP=Itraconazole Nail Patch

Table 3. Evaluation parameter of transungual patches

| Time in hours | FORMULATION CODE | | | | | |
|---|---|---|---|---|---|---|
| | INP1 | INP2 | INP3 | INP4 | INP5 | INP6 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 12.633 | 13.778 | 13.612 | 15.789 | 11.088 | 13.451 |
| 2 | 19.577 | 23.558 | 20.817 | 26.802 | 15.817 | 17.644 |
| 4 | 24.706 | 34.673 | 33.368 | 39.159 | 21.341 | 22.794 |
| 6 | 36.727 | 44.856 | 40.557 | 46.239 | 28.398 | 34.987 |
| 8 | 42.976 | 56.567 | 53.782 | 58.404 | 36.938 | 45.425 |
| 10 | 56.053 | 63.893 | 63.322 | 66.292 | 53.004 | 56.127 |
| 12 | 64.916 | 72.065 | 70.186 | 74.719 | 62.863 | 67.131 |

Table 4. Cumulative % drug release data of Transungual patches INP-1 to INP 6

ISSN No:-2456-2165

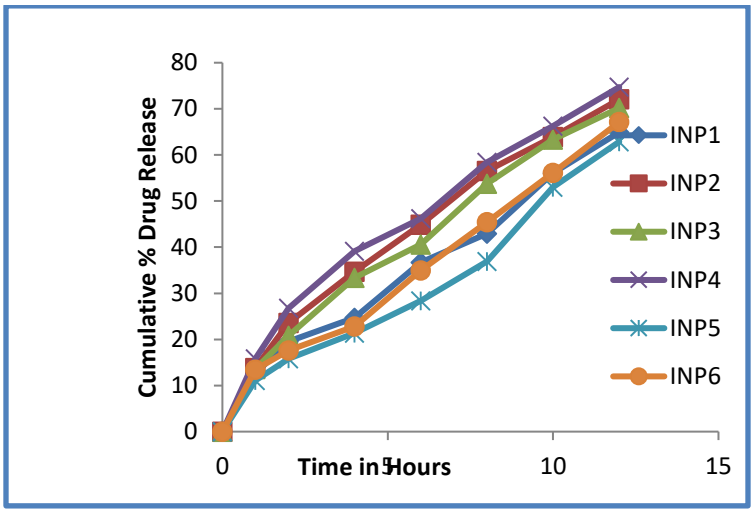


Fig 6:- Cumulative % drug release plot of Trans ungual patches INP-1 to INP-6

ISSN No:-2456-2165

## VIII. CONCULSION

All the formulated patches that evaluated shows good physiochemical properties with respect to their thickness,weight variation,folding endurance,moisture gain,moisture loss and optimum cumulative % drug release from transungual patches of antifungal drug Itraconazole. All the evaluated parameter results shows promising results.Permeability effect of permeation enhancers like oleic acid, propylene glycol, PEG400 has been evaluated by in-vitro permeation studies of formulatd tranungual patches and the results was found to be effective.From these studies it has been understood that as the concentration of penetration enhancer increases,drug permeation was shown to be increased. This results proved that the problems of Itraconazole on oral administration This result revealed that the problems of Itraconazole on oral administration like absorption of drug is rate determined by dissolution rate,nausea/vomiting ,diarrhea,headache,dizziness,stomach upset and other gastric side effects can be overcome by administration of antifungal drug in the form of transungual patch with incorporation of suitable penetration enhancers instead of topical therapies. From the above observations it can be concluded that the present research work has scope for further invivo evaluation (pharmacokinetic and pharmacodynamicstudy).

ISSN No:-2456-2165

## IX. ACKNOWLEDGEMENTS

The authors wish to thank: Itraconazole was kindly gifted from Hetero Pharma Ltd,Hyderabad,, India. And also thankful to management of MLR Institute of Pharmacy,Dundigal, Hyderabad for providing all the facilities for carried out research work.

## REFERENCES

[1]. The human nail:structure,growth & pathological changes.Current problem in dermatology.9,1981,page no.,102-110.
[2]. Manvi F.V and Dandagi,P.M,Gada,A.P.,Mastiholimath,V.S.and Jagadeesh,Indian J.Pharma.239-243,2003.
[3]. Kavitha K and Mangesh Rajendra,Patel Dipen,Mangaibai,SAndeep DS, Ganesh N S,international journal of pharma and biosciences,vol-2,2011.
[4]. Prabhakara, Sreekanth J, Jayaveera K N, *International Journal of Pharmaceutical Science & Research*, 1, (3), 259-266. 2 Runne, U.
[5]. VBRajendra:ABaro:AKumari:DLDhamacha:SRLahoti:SD shelke.J.APPL.Pharm.Sci,2012,2910,203-209.
[6]. MA Ghannoum:RA Hajjeh:R Scher.J.Am.Acad.Dermatol.,2000,43,641-648.
[7]. R Kaur:B KASHYAP:P BHALLA. Ind.j.Med.Micobiol.,2008,26,108-116.
[8]. A Shemer . Dermatol.ther.,2012,25,582-593.
[9]. R Elkeeb:X Hui:N Murthy:HI Maibach:Expert opin.Emerg.drugs,2014,19(4),489-495.
[10]. DH Singh :S Roychowhdhury:D Sharma:V Bhandari:M Singh. Indo Amer .J.Pharma.Res.,2013,3(6),4460-4470.
[11]. Cashman, M.W. and Sloan, S.B. 2010 and Stenn K. and Fleckman, P., (2000).
[12]. Murdan S. 2008 and Conejo-Mir, J. and Requena, L.( 2007).
[13]. Dykyj,J.D.Anotomy of the nail clipadia .med.surgery.
[14]. Cashman, M.W. and Sloan, S.B. ( 2010) and Picardo, M. et al 1994.
[15]. Forslind,B.On the structure of the normal nail.Ascanning electron microscopy study.
[16]. Rania Elkeeb, Ali Alikhan, Laila Elkeeb's, Transungual drug delivery:Current status, IJP, 2010,pg no:1-8.
[17]. RK,Tavakol A,sigurgeirsson Br,HayRJ,Joseph WS,Tosti,Onychomycosis.
[18]. H. N. Shivakumar. Murthy Narashima. Transungual drug delivery department of pharmaceutics, university of Mississippi, 2006; 4: 92-94.
[19]. S Firoz,MN Sirisha,R Rajalakshmi..Int.J.Innovative Drug Delivery ,2011,1(1),9-14.
[20]. MURDAN S (2002),Explained the "Enhancing the nail permeability of topically applied drugs",Expert Opinion Drug Delivery".