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Development, Optimization of Microemulsion Based Transdermal Gel for Antifungal Drug

Yadav Shiva Jogendra*, Manjunath Uddhavrao Machale, Sadhana Jaiswar

Department of Pharmaceutics, Oxbridge College of Pharmacy, Bangalore-91, Karnataka

A b s t r a c t

A new oil-in-water microemulsion-based (ME) gel containing 1% itraconazole (ITZ) was developed for topical delivery. The solubility of ITZ in oils and surfactants was evaluated to identify potential excipients. The optimized microemulsion was characterized for its morphology and particle size distribution. The optimized microemulsion was incorporated into polymeric gels of guar gum and Xanthan gum for convenient application and evaluated for pH, drug content, viscosity, and Spreadability, homogeneity. Furthermore, in vitro antimycotic inhibitory activity of the gels was conducted using a modified. Franz diffusion cell and dialysis membrane. The pH of all the gel formulations was found within the neutral pH range which is compatible with skin. And the viscosity of the formulations was found to be feasible for topical drug delivery. The drug content of the three formulations was found in the range of 81.23% to 99.24% which shows efficient drug loading. Results of In vitro drug release study showed that F8 formulation has better diffusion of drug through membrane. The compatibility study showed that the major peaks in FTIR spectra of the pure drug were found to be intact in their physical mixture. Hence there is no interaction between drug and Xanthum gum, guar gum in their physical mixture. Xanthum gum, guar gum can be effectively used as the polymer for topical gel preparation. And F8 formulation containing 1.5 % w/w Xanthum gum and 0.75% guar gum may be effectively used as topical transdermal delivery for itraconazole.

Keywords: Itraconazole, Transdermal Gel, Drug release, Compatibility study HCl, microemulsion,HPMC,Carbopol940.

A r t i c l e I n f o

***Corresponding Author**
Yadav Shiva Jogendra
Department of Pharmaceutics,
Oxbridge College of Pharmacy,
Bangalore-91, Karnataka

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C o n t e n t s

1. Introduction

Microemulsion solutions gained recognition in 1943 after Hoar and Schulman mixed a milky solution with hexanol to produce a uniform single-phase, non-conducting solution. The first commercial application of microemulsions was in the formulation of liquid waxes, discovered by rodawald in 1928. Microemulsions are transparent, thermodynamically stable mixtures of oil and water stabilized by emulsifiers. They have significantly different properties (type, size, formation and stability) relative to nano and macroemulsions. Microemulsions are uniquely equipped for drug delivery. In particular, microemulsions are able to: i) administer APIs in liquid form, ii) improve bioavailability and stability via small droplet sizes, iii) solubilize and delivery both hydrophilic and lipophilic drugs, form spontaneously with relatively simple starting ingredients. The microemulsion or nanoemulsion used for a thermodynamically stable or kinetically stable, clear dispersion of two liquid phases in which one is water, and other is oil; stabilized by an interfacial film of surfactant and co-surfactant. The microemulsion is a versatile carrier

with their various remarkable properties like enhanced bioavailability of the high absorption, and permeation because of very low surface tension and small droplet size as well as cost-effective approach(2). Microemulsion (ME), as a colloidal nanosize carrier system, has emerged as a promising vehicle for topical administration of drug with poor water solubility, and its potential for drug percutaneous delivery was mainly attributed to the properties of having excellent solubilizing capacity and being penetration enhancers

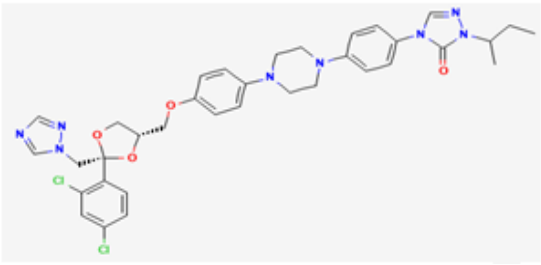

Figure.1

Description: Itraconazole is white to off-white powder
Chemical structure: 2-butan-2-yl-4-[4-[4-[4-[[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4yl]methoxy]phenyl]piperazin-1-yl]phenyl]-1,2,4-triazol-3-one
Formula: $C_{35}H_{38}Cl_2N_8O_4$
Molecular mass: 705.6
Category: Antifungal
Solubility: Practically insoluble in water and dilute acidic solutions
Mechanism of action: The mechanism of action of itraconazole is the same as the other azole antifungals: it inhibits the fungal-mediated synthesis of ergosterol, via inhibition of lanosterol 14α-demethylase.
Polymer profile:
Hydroxy Propyl Methyl Cellulose (HPMC)
Synonyms: Cellulose, Hydroxy propyl methyl ether, HPMC, Propylene glycol Ether; Methocel, Pharmacoat, Methyl cellulose, Methyl hydroxy propyl cellulose.

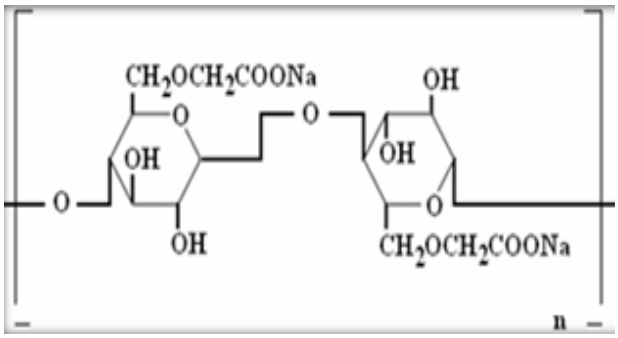

Figure.2

Chemical Name: Cellulose, 2-hyfroxypropyl methyl ether.
Molecularweight:10,000-15,00,000
Category: Coating agent, film-former, stabilizing agent, suspending agent, tablet binder, and viscosity increasingagent.
Description:
Hydroxy propyl methyl cellulose is an odorless, tasteless, white or creamy-white colored fibrous or granular powder.
Solubility: Soluble in cold water, insoluble in chloroform, ethanol and ether, but soluble in mixtures of ethanol or methanol and Dichloromethane
Stability and storage:
It is a stable material although it is hygroscopic after drying. Increase in temperature reduces the viscosity of solutions. It undergoes a reversible solution to gel transformation upon heating and cooling respectively. The powder should bestoredin a well-closed container in a cool dryplace.

2. Materials and Methods

Table.1. List of chemicals used

S.NO	Materials used	Company
1.	Itraconozole	Cipla Pvt. Ltd., Mumbai
2.	Carbopol 936	Loba chemiePvt.Ltd.Mumbai.
3.	HPMC	Loba chemiePvt.Ltd.Mumbai.
4.	Liquid paraffin	Ontop Pharmaceutical Pvt. Ltd. Banglore.
5.	Tween 80	Loba chemiePvt.Ltd.Mumbai.
6.	Span 20	Loba chemiePvt.Ltd.Mumbai.
7.	Ethanol	Loba Chemie, Mumbai.
8.	Propyl paraben	Fisher Scientific, Mumbai.
9.	Guar Gum	SD Fine Chem.pvt Ltd
10.	Xanthum Gum	SD Fine Chempvt Ltd

Method:
The method only differed in the process of making gel in different formulations. The preparation of emulsion was same in all the formulations. The gel formulations were prepared by dispersing xanthan gum and guar gum in purified water with constant stirring at a moderate speed. The oil phase of the emulsion was prepared by dissolving Span 20 in light liquid paraffin while the aqueous phase was prepared by dissolving Tween 20 in purified water. Preservatives were dissolved in propylene glycol, whereas drug (Itraconazole) was dissolved in ethanol and both solutions were mixed with the aqueous phase. Both the oily and aqueous phases were separately heated to 70°–80°C; then, the oily phase was added to the aqueous phase with continuous stirring until cooled to room temperature.
Evaluation
Determination of solubility of drug:
Solubility is a chemical property in which solute dissolved in a solvent. It observed when maximum amount of solute dissolved in a solvent at equilibrium. Solubility depends on the nature of drugs as well as the solvent. Polar solutes dissolved in polar solvent and non-polar solvents dissolved only non-polar solutes. The nature of the solvent can affect the solubility of drugs. A state of dynamic equilibrium established between these two processes and at this point, the number of solute molecules enters in the solution and becomes equal to the number of particles leaving the

solution, concentration of the solute in the solution remains constant at a given temperature and pressure conditions. A solution which have no more capacity to dissolve more solute in the solvent at a given temperature and pressure called saturated solution. Solubility profile of itraconazole is carried out in the various solvents like ethanol, methanol acetone and buffers.

Determination of Aqueous solubility of itraconazole:

The determination of aqueous solubility of itraconazole estimated through Saturation shake - flask method. An optimum amount of itraconazole dissolved in distilled water and phosphate buffer pH 7.4 then followed by vortex and centrifugation at 50rpm at 37°C for 48hrs, resulting solution filtered and analyzed spectrophotometrically at 273 nm. Qualification is taken in triplicate.

Determination of partition co-effiecient:

The partition coefficient of itraconazole determined using n-octanol and water partition system. Measured amount of itraconazole placed inside conical flask containing measured volumes of an n-octanol and aqueous buffer solution. flask shaken with uniform time interval for 48h to attain equilibrium and then resulting mixture placed to separating flask with final shaking and kept remains undisturbed to be separated inside two layers. Targeted measurement proceeded to be analyzed spectrophotometrically at 273 nm. Resulting values of both phases were determined in form of log P of ratio calculated.

Determination of λmax.

A solution of itraconazole containing conc. 10µg/ml was prepared and UV- Spectrum was taken using Shimadzu (UV- 1800) spectrophotometer. The solution was scanned in the range of 200-400nm.

Standard calibration of Itraconazole:

Accurately weighed 100 mg of Itraconazole was dissolved in 100 ml of 7.4 Phosphate buffer (Standard Stock solution). From the above solution 10 ml was pipetted and diluted to 100 ml with & 7.4 buffer (Stock I). From this solution aliquots were prepared of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 ml into 10 ml volumetric flask with phosphate buffer 7.4 to satisfy the Beer's range of 5-50 µg/ml. Absorbance read at UV-VIS Spectrophotometer at 273nm against blank (phosphate buffer 7.4). The calibration curves of Itraconazole.

Physical appearance:

The prepared emulgels are visualized for their colour, homogeneity and phase uniformity.

Determination of pH:

Pre-calibrated Eutech pH tutor was used to analyze and adjust the pH of the all the emulgel formulations). 2.5g of gel was dispersed or mixed in 25ml of purified water. The value of pH was determined at pH meter.

Procedure:

The glass electrode of the pH meter was cleaned with purified water and blotted dry using a tissue paper. Then the electrode was directly immersed into the sample taken in a borosil beaker and the pH was determined at an ambient temperature of 25 ± 0.2oC. If required the preparations of all gels was set to a pH to around 6.2-6.7 using pH modifiers.

Homogeneity:

All formulated emulgels are visualized for their homogeneity to clarify whether there was any grittiness.

Viscosity:

All the formulations were subjected to the viscosity determination using brook field viscometer using spindle no 04. The spindle was dipped perpendicular to the center of formulation and rotated at the speed of 2.5 rpm for 5 min.

Spreadability:

10gms of sample was placed in the spreadability set up and about 50 gm of weight was loaded on the upper slide and pulled by 80 gm of sample. The time taken to move the sample was written and it is done in triplicate. It is calculated by the formula

$$S = ML/T$$

Where, S is the spredability of gel formulations, 'm' is the weight (g) tied to upper slide (80gm) L is the length of glass slide (6cm) and T is the time in seconds.

FT-IR study:

Attenuated total reflection-Fourier transform infrared spectrometry (ATR-FTIR) Infrared spectra of emulgel formulations were recorded in Bruker ATR alpha kept at a ambient temperature of 25.0 ± 0.5oC. The analytical procedure was simple and did not need any special sample preparation. The spectra were recorded by scanning the samples in region of 4000-400 cm-1 to determine various functional groups. The IR spectra drug with excipients were compared with that of pure drug to check for any possible drug excipients interaction and confirm chemical integrity of itraconazole in the microemulsion.

Stability Studies:

Stability studies of pharmaceutical products were done as per ICH guidelines. These studies are designed to increase the rate of chemical or physical degradation of the drug substance or product by using exaggerated storage conditions.

Method:

In order to determine the change in *in-vitro* release profile and drug content on storage, stability study of formulation and optimized formulation was carried out at 40°C in a humidity chamber having 75% RH. The samples were withdrawn after a period of 15 days, 30 days, 60 days and 90 days. Formulation 8 was evaluated for in vitro drug release profiles.

DSC Studies:

Differential scanning calorimetry (DSC) is a thermo analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment.

SEM Imaging

The powders were imaged by a scanning electron microscope (SEM) run at an accelerating voltage of 10kV using Hitachi S 3700. The powder in few µg were fixed on to stub by a double sided sticky carbon tape and kept inside the SEM chamber and analyzed at different magnification such as to obtain better clarity on the particle morphology/ topology.

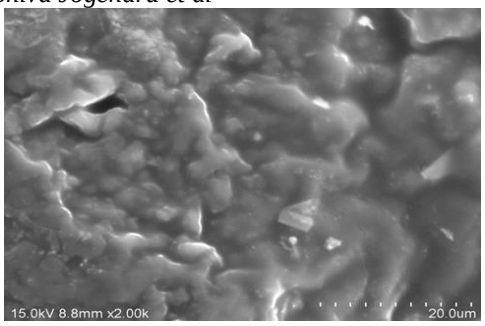

Figure.3. SEM image of itraconazole

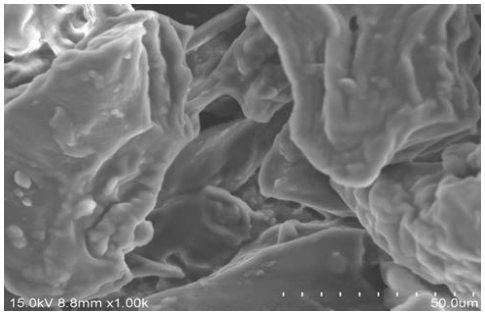

Figure.4. SEM image of itraconazole

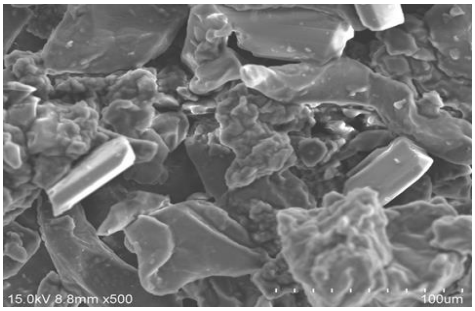

Figure.5. SEM image of itraconazole

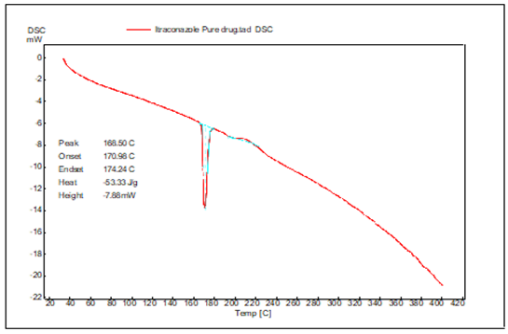

Figure.6. DSC Graph

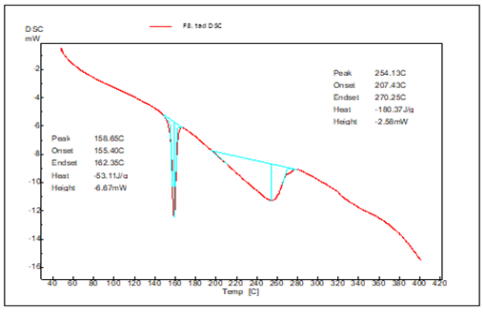

Figure.7. DSC graph

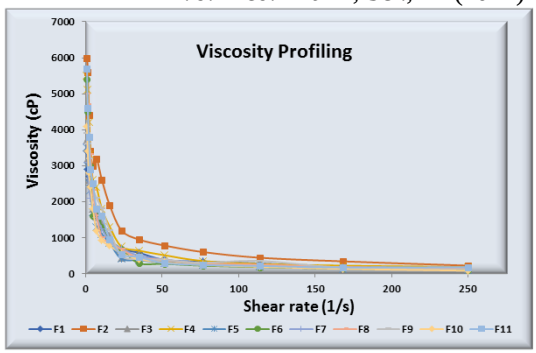

Figure.8. Viscosity profile

3. Results and Discussion

Formulation:

Emulsion based gels (Emulgels) of itraconazole were developed with a view to deliver the drug on topical surface of skin. Analytical method was developed, preformulation studies were conducted, formulations were developed by use of Design expert, and stability studies were conducted. MS-Excel and MS Word were used for calculations including graphs and word processing, respectively. A Core i3 500 gb Hard disk laptop was used. The details of results and discussion are given in the following sections.

Preformulation Studies

The following preformulation studies were performed on itraconazole and excipients.

Melting Point

Melting point of itraconazole was determined by open capillary method and it was found to be 166.33 ± 0.763ºC (*n* = 3). This value is same as that of the literature citation of 166.2ºC.

Determination of solubility and partition co efficient of itraconazole:

Physicochemical studies of itraconazole were conducted to evaluate the physicochemical properties of drug. Studies were conducted to evaluate itraconazole hydrophilic and lipophilic compatibility. Result shows that itraconazolehaving poor solubility potential with water which found to be 1.2±0.03µg/ml, and Log P value of itraconazole in water and n-octanol obtained is 5.4 that correlated with the published data.

Drug–Polymer Compatibility Studies

As described in the methodology section the FT-IR studies were carried out for pure drug alone and along with polymers. The results are summarized as follows. An FT-IR spectrum of pure itraconazole is shown in the Figure. Similarly FT-IR spectra of itraconazole in combination with polymers (xanthum gum and guar gum) are shown in Figures.

The peaks observed in Figure 03 are can be considered as characteristic peaks of itraconazole. These peaks were compared with the peaks obtained from the Figures 04 to 07. It was observed that the peaks were not affected and were prominently observed in FT-IR spectra given in Figures 03 to 07. This indicates that there is no interaction between itraconazole and polymers and the drug was compatible with the formulation components.

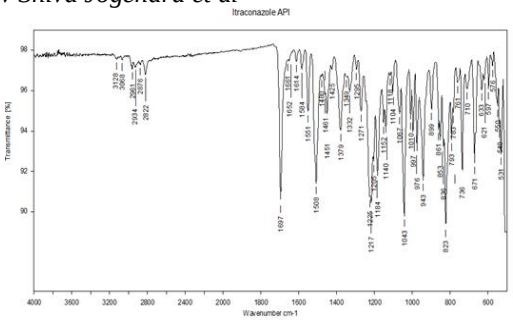

Figure 9: FT-IR spectrum of itraconazole

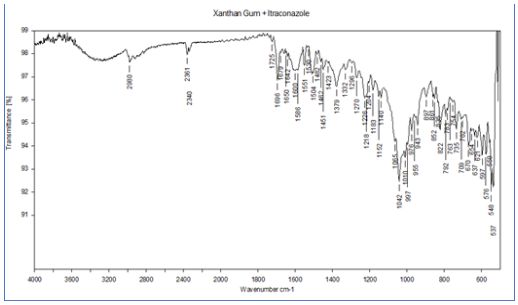

Figure 10: FT-IR spectrum of physical mixture of Xanthum gum and itraconazole

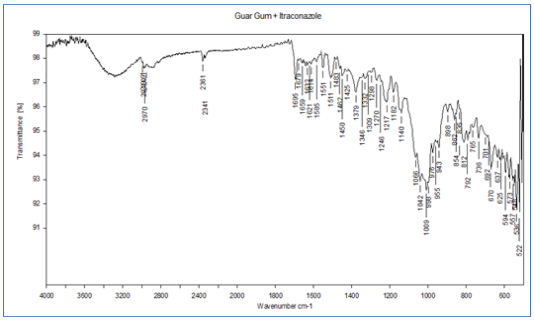

Figure 11: FT-IR spectrum of physical mixture of guar gum and itraconazole

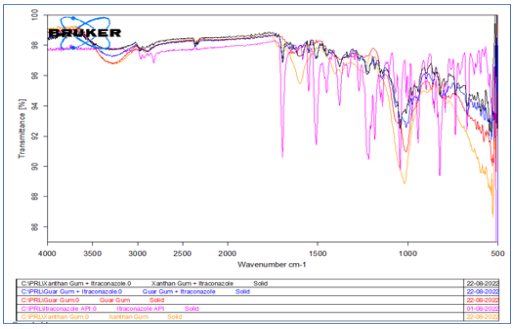

Figure 12: FT-IR spectrum of physical mixture of microemulsion gel of itraconazole

Microemulsion:

Development of the formulation in the present study was mainly based on the type of polymer, combination of polymers, and the drug. Two polymers and excipients in different combinations (based on central composite design by Design expert 13) were used to prepare emulgels. Itraconazole is water insoluble drug and made soluble using ethanol and the solution is used for the preparation of emulgel and release profile of the emulgels were studied.

Operational Concept of Emulgels

Before describing the formulation aspects of emulgel, it is necessary to understand the intended operational aspects of the emulgel. In a majority of cases, emulsions(o/w or w/o types) are unstable and are made most stable form by the addition of gelling agents.

Manufacture of Emulgels

As described in the methodology chapter the emulgels were prepared by simple method. In this method, all the concentration of gums are varies according to central composite method. Tween 20 and span 20 acted as surfactants and parabens were used as preservatives. Liquid paraffin used as oily phase in formulation of emulgels. 1% w/w of drug used in all the formulation as 1% w/w of marketed gels are available.

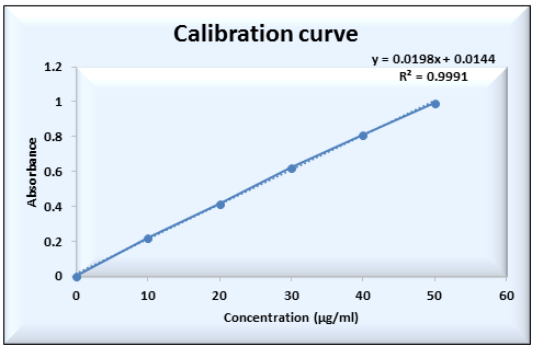

Figure 13

In vitro Diffusion studies profile for the batches F-1 to F11: After getting all the parameters satisfactory for batches F1 to F11, the dissolution studies were conducted. Diffusion studies were carried out as per the procedure mentioned in methodology chapter. The details of the diffusion study for the emulgels of the batches F-1 to F-11 are given in the Table 6.2.3 and the Figure 6.2.6.The emulgels of F-I, to FXI showed 86 to 97% of drug release in 180 min, respectively. Drug release profile of all these formulations did not show 100 % drug release within 180 minutes may due to various factors of polymers and polymeric network formed during the formulations.

Table 2. Formulation of ITZ gel

Ingredients % w/w	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Itraconozole	1	1	1	1	1	1	1	1	1	1	1
Xanthum gum	1.66	1.12	1.12	0.59	1.12	0.75	0.75	1.5	1.12	1.12	1.5
Guar gum	1.12	1.66	1.12	1.12	1.12	1.5	0.75	0.75	1.12	0.59	1.5
Liquid paraffin	6	6	6	6	6	6	6	6	6	6	6
Tween 20	1	1	1	1	1	1	1	1	1	1	1
Span 20	2	2	2	2	2	2	2	2	2s	2	2

Propylene glycol	8	8	8	8	8	8	8	8	8	8	8
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Ethanol	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Water	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS	QS

Table 3.Physical properties of the microemulsion gel from F1 to F11

Formulations	pH	Spreadability (g.cm/sec)	Extrudability (g.cm/sec)	Drug content (%)
F1	6.42±0.024	30±0.113	55±0.152	96.18±0.133
F2	6.20±0.027	45±0.884	38±0.174	92.36±0.104
F3	6.53±0.122	21±0.350	44±0.366	95.19±0.179
F4	6.51±0.134	36±0.361	46±0.300	93.55±0.244
F5	6.78±0.081	46±0.438	48±0.351	95.28±0.135
F6	6.35±0.014	53±0.382	50±0.438	96.60±0.175
F7	6.35±0.028	52±0.029	50±0.728	86.33±0.053
F8	6.85±0.084	25±0.293	78±0.694	99.24±0.290
F9	6.44±0.164	33±0.303	76±0.385	91.79±0.141
F10	6.56±0.088	36±0.217	65±0.180	85.56±0.184
F11	6.69±0.125	29±0.080	45±0.095	81.23±0.293

Table 4: *In vitro* release of Itraconazole from tablets of F-1 to F-11

Time in min	% Cumulative drug release				
	F1	**F2**	**F3**	**F4**	**F5**
15	15.25±0.104	32.10±0.102	16.20±0.070	21.65±0.080	12.66±0.077
30	35.64±0.398	44.20±0.106	29.21±0.113	33.85±0.104	22.30±0.183
60	44.59±0.102	55.43±0.055	32.10±0.076	48.22±0.115	44.23±0.078
90	62.62±0.108	69.85±0.056	44.50±0.068	56.12±0.082	58.69±0.186
120	78.11±0.096	75.23±0.87	53.44±0.110	63.12±0.110	67.24±0.202
150	89.64±0.137	88.33±0.112	76.20±0.107	89.21±0.059	74.12±0.253
180	94.53±0.092	95.06±0.109	86.78±0.125	91.44±0.099	88.73±0.166

***Each value was an average of three determinations**

Table 5: *In vitro* release of Emulgel of F-8 on 15th, 30th, 60, and 90th Day accelerated stability studies.

Time (hours)	Cumulative percentage of drug released			
	15 Day	**1 Month**	**2 Month**	**3 Month**
0	0	0	0	0
15	23.56±0.040	24.33±0.054	25.11±0.044	24.90±0.051
30	47.94±0.082	48.60±0.065	49.14±0.090	47.81±0.034
60	66.27±0.081	65.92±0.056	68.00±0.089	67.22±0.092
90	73.33±0.102	71.91±0.093	72.34±0.125	72.94±0.108
120	87.12±0.114	87.22±0.090	86.85±0.112	86.34±0.122
150	90.31±0.091	90.63±0.109	91.32±0.113	92.65±0.092
180	96.11±0.106	96.84±0.085	97.61±0.104	96.50±0.078

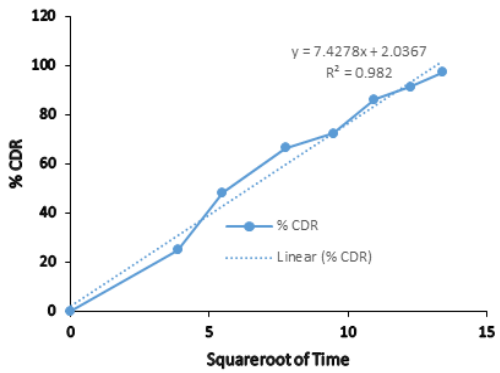

Figure 14. Higuchi Model

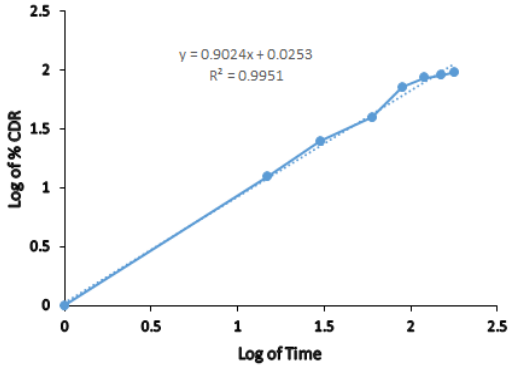

Figure 15. Korsmeyer-Peppas Model

4. Conclusion

Microemulsion is a promising transdermal drug delivery vehicle. An antifungal medication, also known as an antimycotic medication, is a pharmaceutical fungicide or fungistatic used to treat and prevent mycosis such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others. Such drugs are usually obtained by a doctor's prescription, but a few are available over the counter. Microemulsion-containing ITZ was formulated for topical application. Various gel bases containing ITZ microemulsion were studied for drug release, viscosity, primary skin irritation potential, and antimycotic activity of the topical formulations. . As for all drugs, the benefits and risks of treatment should be evaluated carefully for each patient. Microemulsion gel of itraconazole were prepared by simple method. Various evaluation parameters were performed. In previous chapter evaluation and preparation of formulations have been discussed in detail.

5. References

[1] VyasPS, Khar KR, "Drug delivery system", 2012; 2: 397-399.

[2] Robinso JR, Lee HL, "Controlled drug delivery fundamentals and applications", 1987; 2: 524-552.

[3] Misra AN, "Controlled and novel drug delivery" CBS Publishers and Distributors, 1997; 107-109.

[4] Anti kesarwani ,AjitkumarYadav, Sunilsingh, Hemendragautam, Haribansh N singh, et al. (2013)

[5] Aqui SY, Ali A, "Matrix type transdermal drug delivery systems of metoprolol tartrate: In vitro characterization" Acta Pharm, 2003; 119-125.

[6] Singh J, TripathiKP and Sakia TR, "Effect of penetration enhancers on the in vitro transport of ephedrine through rat skin and human epidermis from matrix based transdermal formulations" Drug DevInd Pharm, 1993;1623-1628.

[7] Farra H, LetzigL, Gil M, "Validation of a liquid chromatographic method for the determination of ibuprofen in human plasma"Journal of Chromatography, 2002; 780: 341–348.

[8] Fischer J, Ganellin CR (2006). Analogue-based Drug Discovery. John Wiley & Sons. p. 522. ISBN 9783527607495

[9] Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh, UK: Churchill Livingstone; 2005. p. 666.

[10] Mitchel J. Transdermal drug delivery. Americal academy of dermatology– 58th Annual Meeting, San Francisco, CA, 2000.

[11] Patel V, Kukadiya H, Mashru R, Surti N, Mandal S. Development of microemulsion for solubility enhancement of clopidogrel. Iran J Pharm Res 2010, 9:327-34.

[12] Yamane M, Williams A. Effect of terpenes and oleic acid as skin penetration enhancers. Int J Pharm 1995, 116:237-51.

[13] Touitou E, Godin B, Karl Y, Bujanover S, Becker Y. Oleic acid, a skin penetration enhancer, affects Langerhans cells and corneocytes. J Control Release 2002;80:1-7.

[14] Kawakami K. Microemulsion formulation for enhanced absorption of poorly soluble drugs. J Control Release 2002;81:75-82.

[15] Lawrencea M, Gareth D. Microemulsion-based media as novel drug delivery systems. Adv Drug Deliv Rev 2000;45:89-121.

[16] Singh V, Bushettii SS, Raju AS, Ahmad R, Singh M, Bisht A. Microemulsions as promising delivery systems: A Review. Indian J Pharm Educ Res 2011;45:392-401.

[17] Chandra A, Sharma PK. Microemulsion: An Overview. Pharm Rev 2008;6:24.

[18] Chen H, Moub D. Hydrogel-thickened microemulsion for topical administration of drug molecule at an extremely low concentration. Int J Pharm 2007;341:78-84.

[19] Lackner TE, Clissold SP. Bifonazole. A review of its antimicrobial activity and therapeutic use in superficial mycoses. Drugs 1989;38:204-25.

[20] Sarkar B, Hardenia S. Microemulsion drug delivery system: For oral bioavailability enhancement of glipizide. J Adv Pharm Educ Res 2011;1:195-200.

[21] Dhamankar A, Manwar J, Kumbhar D. The novel formulation design of O/W microemulsion of ketoprofen for improving transdermal absorption. Int J PharmTech Res 2009;1:1449-57.

[22] Naguib G. Microemulsions as vehicles for topical administration of voriconazole: Formulation and in vitro evaluation. Drug DevInd Pharm 2012;38:64-72.

[23] Lee EA, Balakrishnan P, ChngKil Song CK, Choi JH, Noh GY, Park CG, Choi AJ, Chung SJ, Shim CK, Kim DD.Microemulsionbased hydrogel formulation of itraconazole for topical delivery. J Pharm Invest 2010;40:305-11.

[24] Chudasama A, Patel V, Nivsarkar M, Vasu K, Shishoo C. Investigation of microemulsion system for transdermal delivery of itraconazole. J Adv Pharm Technol Res 2011;2:30-8.

[25]. Vicentini FT, Vaz MM, Fonseca YM, Bentley MV, Fonseca MJ. Characterization and stability study of a water-in-oil microemulsion incorporating quercetin. Drug DevInd Pharm 2011;37:47-55. 27. Kantarci G, Ozgüney I, Karasulu HY, Arzik S, GüneriT. Comparison of different water/oil microemulsions containing diclofenac sodium: Preparation, characterization, release rate, skin irritation studies. AAPS Pharm Sci Tech 2007;8:E91. 28. Patel MR, Patel RB, Parikh JR, Solanki AB, Patel BG. Effect of formulation components on the in vitro permeation of microemulsion drug delivery system of fluconazole. AAPS PharmSci Tech 2009, 10:917-23, 29.

[26] Zhua W, Guo C. Microemulsion-based hydrogel formulation of pencyclovir for topical delivery. Int J Pharm 2009, 378:152-8.

[27] Odds FC, Webster CE, Abbott AB. Antifungal relative inhibition factors: BAY 1-9139, bifonazole, butoconazole, isoconazole, itraconazole (R 51211), oxiconazole, sulconazole, terconazole and vibunazole compared in vitro with nine established antifungal agents. J Antimicrob Chemother, 1984;14:105.

[28] Suppaibulsuk B, Prasassarakich P, Rempel GL. Factorial design for nanosizedpolyisoprene synthesis via differential microemulsion polymerization. PolymAdvTechnol 2010;21:467.

[29] Wisplinghoff H, Seifert H, Wenzel RP, Edmond MB. Current trends in the epidemiology of nosocomial bloodstream infections in patients with haematological malignancies and solid neoplasms in hospitals in the USA. Clin Infect Dis 2003; 36: 1103-1110.

[30] Bodey GP, Bueltmann B, Duguid W, Gibbs D, Hanak H, Mall G, Martino P, Meunier F, Milliken S, Naoe S, Okudaira M, Scevola D, van't Wout J. Fungal infections in cancer patients: an international autopsy survey. Eur J Clin Microbiol Infect Dis 1992; 11: 99-109.

[31] Odds FC. The fungal kingdom: essentials of mycology. In: "Principles and Practice of Clinical Mycology", Editors Kibbler CC, Mackenzie WR, Odds FC. John Wiley & Sons. Chicester, England. 1996 ISBN 0471 961043.

[32] Carvalho A, Pasqualotto AC, Pitzurra L, Romani L, Denning DW, Rodrigues F. Polymorphisms in toll-like receptor genes and susceptibility to pulmonary aspergillosis. J Infect Dis. 2008; 197:618-21.

[33] Bochud P-Y, Chien JW, Marr KA, Leisenring WM, Upton A, Janer M, Rodrigues SD, Li S, Hansen JA, Zhao LP, Aderem A, Boeckh M. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. N Engl J Med 2008; 359:1766-1777.

[34] Bow EJ, Loewen R, Cheang MS, Shore TB, Rubinger M, Schachter B. Cytotoxic therapy-induced D-xylose malabsorption and invasive infection during remission-induction therapy for acute myeloid leukemia in adults. J ClinOncol 1997; 15:2254-2261.

[35] Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. Ann Intern Med 1966; 64:328-340.