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Formulation and Evaluation of Nail Lacquer Containing Antifungal Drug

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ABSTRACT

The area between nail and skin are generally affected one that are hard to reach, hence nail drug delivery is interesting and gaining importance. However, the effectiveness of topical therapies is limited by minimal drug permeability through nail plate. Since it is challenging approach to deliver the drug through the nail skin at infected area. The present investigation focuses on the formulation and optimization of medicated nail lacquer containing itraconazole for transungual drug delivery system using nitrocellulose as polymer and two different penetration enhancers (thioglycolic acid and DMSO). The prepared formulation was evaluated for the different parameters like drying time, water resistance, antifungal activity, in vitro diffusion studies and stability studies. Among all formulated batches, A2 batch containing (thioglycolic acid and DMSO) showed better (51.58%) drug release compared to all batches. Findings of the study suggest that combination of permeation enhancers (thioglycolic acid: DMSO 3:6) shows better results compared to other batches. The stability studies of A2 at 40± 2^{o}C5%RH for 2 months does not showed any physical changes during study period and stable at storage conditions. It can concluded that, the nail lacquer formulation containing itraconazole is patient friendly and it is effective dosage form for treating fungal nail infection.

Keywords: Ungual drug deliveries, antifungal nail lacquer, Itraconazole, DMSO, Thioglycolic acid.

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INTRODUCTION

Topical delivery of antifungal drug is highly desirable in treating nail infections due to its localized effects which results delivering drug to desired site which are hard to reach area. Nail lacquer containing azole derivatives in formulation could minimize the side effects of oral dosage forms. [1, 2]The current treatment strategies involve systemic delivery of oral antifungal agents including azole (e.g- itraconazole, voriconazole, miconazole etc), which can be delivered through the nail plate from nail bed. Topical delivery of itraconazole through the human nail offers several advantages over oral therapy.[3]The objective of this research work were to determine the critical factors affecting the delivery of itraconazole across the human nail, to select permeation enhancer(s) specific for itraconazole delivery in to the targeted site. To develop a novel transungual formulation containing itraconazole and permeation enhancer for transungual delivery that could serve a good option in delivering drug to those hard to reach area.

MATERIALS AND METHOD

Selection of polymer and excipients:

Nitrocellulose was selected as polymer on the basis of their film forming properties and adhesiveness. Thioglycolic acid and dimethyl sulfoxide are used to enhance permeability of drug through nail skin. Dibutyl phthalate was used as a plasticizer. Solvent mixture (isopropyl alcohol, ethyl acetate, toluene) used to solubilize the polymer and smoothness of film.[4,5]

UV-visible spectrophotometric characterization of itraconazole (λmax determination)

Preparation of stock solution

Accurately weighed 100mg of itraconazole was transferred to a 100ml volumetric flask containing 50ml methanol and the mixture was sonicated for 30min then diluted to 100ml with methanol (1000µg/ml). The solution was filtered and 1ml of filtered solution diluted to 10ml of methanol giving the stock solution of 100µg/ml.[6]

Preparation of serial dilution:

From the stock solution, aliquots of 0.4 to 1.4ml were transferred to the 10ml volumetric flask and final volume was made to 10ml with methanol to get 4-14µg/ml concentration respectively. Finally the absorbance's of prepared solutions were measured against blank (methanol) at 261 nm by using UV visible spectrophotometer and calibration curve was plotted.[6]

Difference scanning calorimetry (DSC)

DSC was performed in order to assess the thermotropic properties and thermal behavior of itraconazole. About 5mg of the sample was sealed in the aluminium pans and heated at the rate

of 10 per minute covering a temperature range of 40 to300 under nitrogen atmosphere of flow rate 100ml/min. [6]

Infrared spectral (FTIR) analysis:

Fourier Transform Infrared (FTIR) spectroscopic analysis of itraconazole was carried out to assess its purity. The Infrared spectrum of itraconazole was taken on FTIR spectrophotometer (Alpha-E, Bruker). Approximately 10 mg of sample was required. The sample was analyzed in the range of 600 cm^{-1} to 4000 cm^{-1}. The obtained FTIR spectrum was then studied and compared with standard. [7]

Formulation development of nail lacquer:

Preparation of Itraconazole nails lacquer:

Nail lacquer was prepared by using simple mixing method. Nail lacquer was composed of 1% drug, 3% nitrocellulose, 25% mixture of solvents and 1% plasticizer.

Factorial Design:

A factorial design is used to evaluate two or more factors simultaneously. The treatments are combination of level of the factors. A factor is simply a categorical variable with two or more values, referred to as levels. A study, in which there are 2 factors with 3 levels, is called as 3^2 Factorial Design. A 3^2 full factorial design (FFD) was constructed where the amounts of thioglycolic acid (X_1) and DMSO (X_2) were selected as the factors. The levels of the two factors were selected on the basis of the preliminary study carried out before implementing the experimental design. All other formulation and processing variables were kept invariant throughout the study. [8]

Table 1: Composition of nail lacquer

Sr. No.	Ingredient	Use
1	Itraconazole	Drug
2	Nitrocellulose	Polymer
3	Thioglycolic acid	Permeation enhancer
4	Dimethyl sulfoxide (DMSO)	Permeation enhancer
5	Isopropyl alcohol	Solvent
6	Toluene	Solvent
7	Ethyl acetate	Solvent
8	Dibutylphthalate	Plasticizer

Table 2 Composition of nail lacquer containing itraconazole

Ingredients	Formulation								
	A1	A2	A3	A4	A5	A6	A7	A8	A9
Itraconazole	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm
Nitrocellulose	2gm	2gm	2gm	2gm	2gm	2gm	2gm	2gm	2gm
Isopropyl alcohol	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml

Ethyl acetate	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml
Toluene	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml
Thioglycolic acid	0.3ml	0.3ml	0.3ml	0.5ml	0.5ml	0.5ml	0.7ml	0.7ml	0.7ml
DMSO	0.4ml	0.5ml	0.6ml	0.4ml	0.5ml	0.6ml	0.4ml	0.5ml	0.6ml
Dibutyl pthlate	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml

Evaluation of nail lacquer:

Nonvolatile content:

1gm of sample was taken in a glass petridish of about 8 cm in diameter. Sample was spread evenly with the help of tared wire. The dish was placed in the oven at 105^{o}C for 1hr the petridish was removed, cooled, and weighed. The difference in weight of sample after drying was determined. [8, 9]

Drying time:

A film of sample was applied on a glass petridish with the help of brush. The time to form a dry to touch film was noted using a stopwatch. [8]

Water resistance

This is the measure of the resistance towards water permeability of the film. This was done by applying a continuous film on a surface and immersing it in water. The weight before and after immersion was noted and increase in weight lower the water resistance. [9]

Drug content

Drug content of nail lacquer was determined by dissolving accurately 1ml of nail lacquer in methanol. After suitable dilution absorbance was recorded by using UV- visible spectrophotometer (UV- 1700, Shimadzu, Japan) at 260nm. [9]

Diffusion studies across artificial membrane

Diffusion studies were performed using artificial membrane (cellophane). The membrane was soaked for 1hr in solvent system (phosphate buffer, pH 7.4), and the receptor compartment was filled with solvent. Test vehicle equivalent to 10mg was applied evenly on the surface of the membrane. The whole assembly was maintained at 37^{o} C, and the speed stirring was kept constant (600 rpm) for 10hrs. The 5ml aliquot of drug sample was taken after time interval of 1hr and was replaced by fresh solvent. Each experiment was replicated thrice. The drug analysis was done using double beam UV spectrophotometer (U.V. 1700 Shimadzu Corporation). [9]

Antifungal activity:

The antifungal activity of nail lacquer containing itraconazole was carried out by using cup plate method. The fungi culture used for this activity was *Candida albicans*.[9]

Stability studies:

Stability studies were carried out at 40± 2°C /75 ±5% RH for two months; sample was stored in stability chamber. The sample was evaluated for nonvolatile content, drying time, gloss, and smoothness of flow, water resistance and diffusion across artificial membrane. [10, 11]

RESULTS AND DISCUSSION

Characterization of pure drug:

Melting point:

Melting point of itraconazole was found to be in range of 158.2- 166.5° C, while as per literature standard it is reported to be 166.2°C. The melting point of drug was found to be significantly comparable with that given in literature.

Purity of drug:

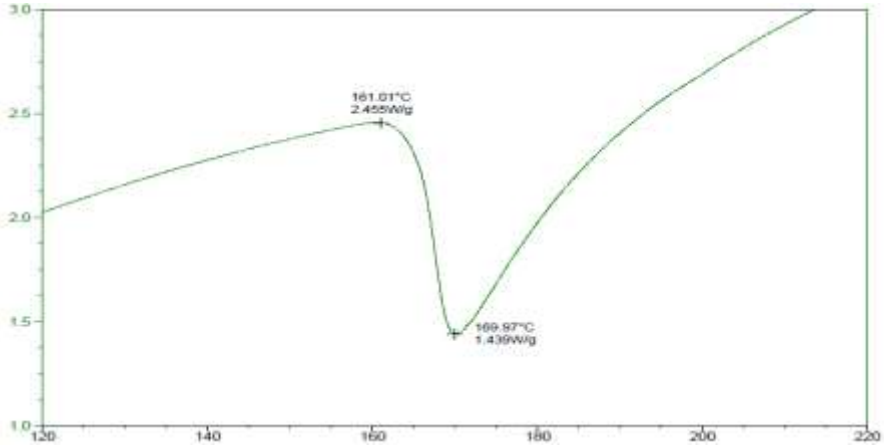

Figure 1: Differential Scanning Calorimetry-

According to the thermo gram, a sharp endothermic peak was observed at 169.50°C which corresponds to the melting point of pure drug. DSC graph show that purity of drug.

FTIR Spectroscopy:

The infrared spectrum of itraconazole was recorded and spectral analysis was done using FTIR spectroscopy which is shown in figure 2.

Table 3: IR interpretation of Itraconazole

Sr.No.	Wavenumber(cm-1)	Functional Group Associated
1	1695.52	C=O-N-H
2	1443	C-N stretching
3	1379	CH_3 Group
5	3000-3600	Overtone (aromatic compound)

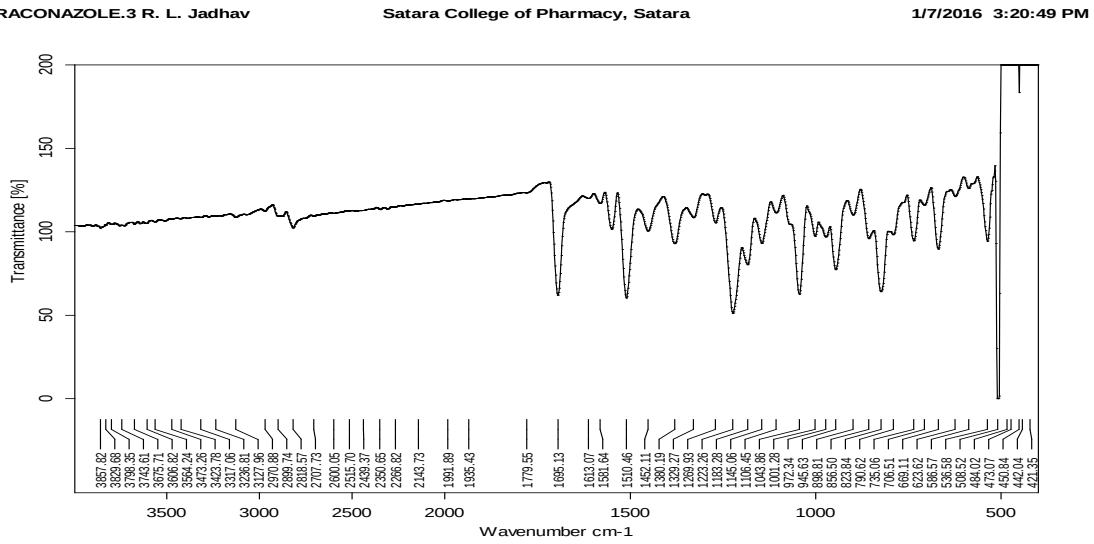

Figure 2: Characteristics peak of FTIR Spectrum of Itraconazole.

The infrared spectral analysis of the procured Itraconazole was compared with standard IR spectrum of Itraconazole. In the course of IR study, it was found that all the important characteristic peak were present, which confirmed the purity of drug sample.

UV Spectroscopy:

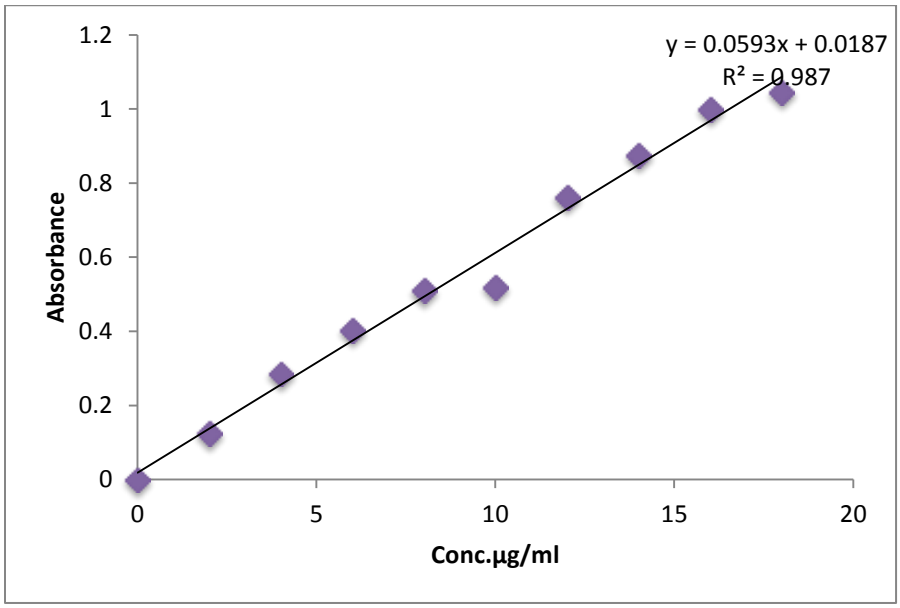

Figure 3: Calibration curve of Itraconazole in ethanol

Evaluation studies:

Non volatile content of nail lacquer:

It was observed that as the polymer concentration is kept constant i.e. 12% w/v. the formulation which had optimum concentration of polymer showed higher nonvolatile content. As the amount of polymer present in the sample for determination of nonvolatile content was more as compared

to the formulation which contained lower concentration of polymer. The non volatile content values for all the formulations are given in table no.4.

Table 4: Non volatile content of nail lacquer

Formulation	Non volatile content (%)
A1	41.2± 0.57
A2	38.5± 0.72
A3	42.6± 0.50
A4	41.3± 0.57
A5	37.2±0.72
A6	43.3±0.57
A7	38.5±0.72
A8	41.3±0.57
A9	37.2±0.72

(Average of three trials (n=3))

Drying time:

The drying time for all 9 formulations was found to be in the range of 72 to 136seconds. As the concentration of permeation enhancer increases the drying time increases. As the drying time increases the drug retained for longer period and the drug retained at targeted site and get permeated across nail skin.

Water resistance test for nail lacquer:

In water resistance test it was found that as the polymer concentration was optimum (2gm kept constant for all batches) water resistance is also optimum. As the polymer concentration increases the water resistance also increases i.e. the amount of water absorbed by the nail lacquer film after keeping in water for 24 hours is less. The value of amount of difference in weight of nail lacquer film before and after keeping in water for 24hrs is given in table

The drug content was found to be in range of 96% to 99% and hence there was a good uniformity of drug content in the all the formulations.

Table 5: Water resistance test for nail lacquer

Formulation	W1(g)	W2(g)	W1-w2(g)
A1	8.00	8.11	0.11
A2	8.00	8.21	0.21
A3	8.00	8.10	0.10
A4	8.00	8.12	0.12
A5	8.00	8.22	0.22
A6	8.00	8.24	0.24
A7	8.00	8.22	0.22
A8	8.00	8.12	0.12
A9	8.00	8.16	0.16

FTIR spectrum of formulation:

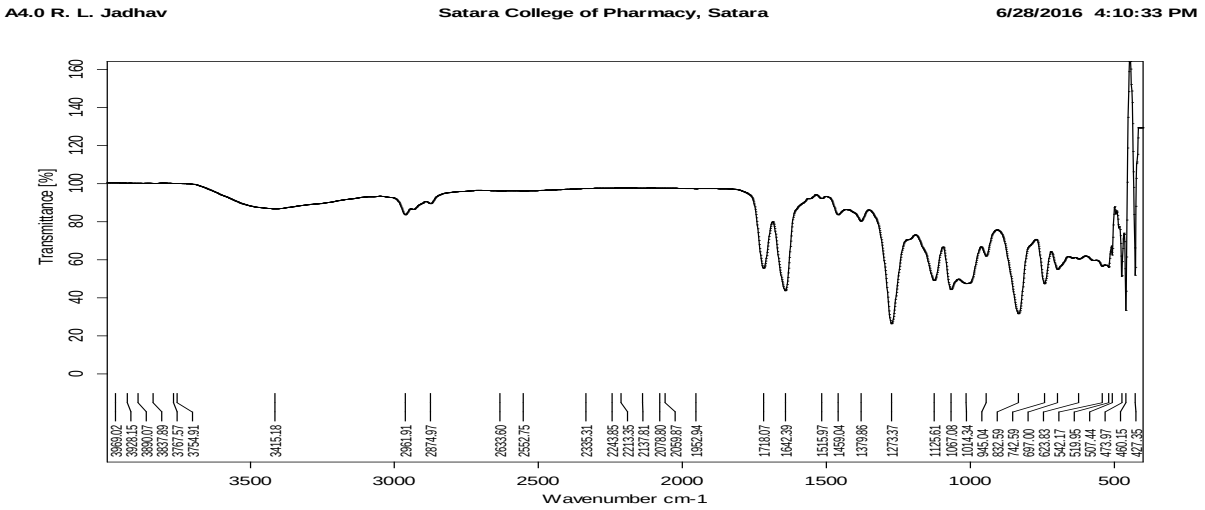

Figure 4: FTIR spectrum of formulation (batch A4)

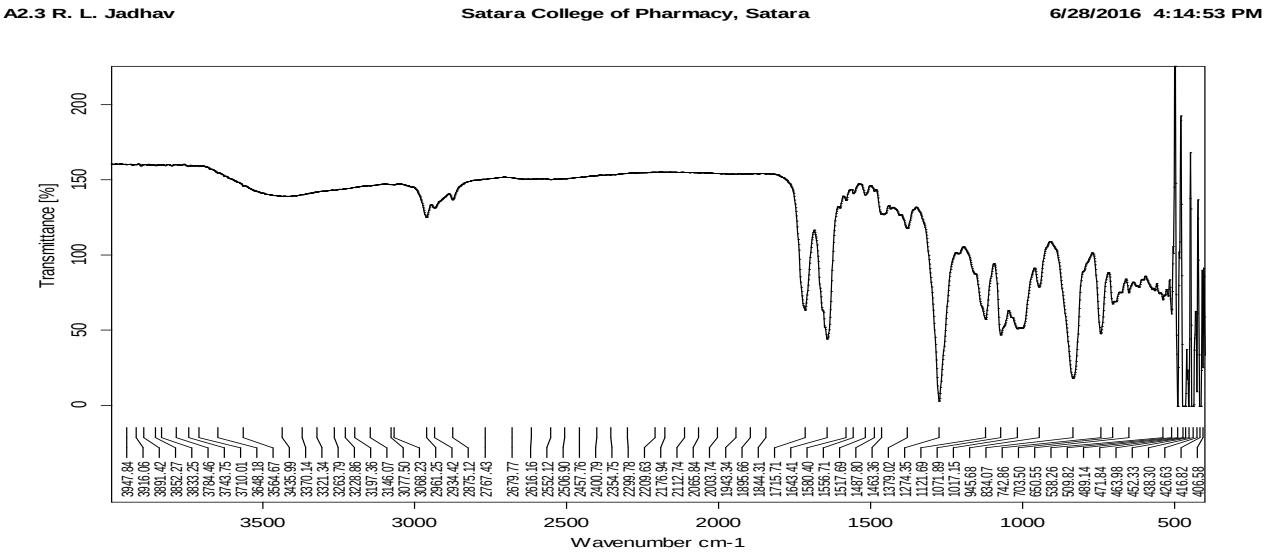

Figure 5: FTIR spectrum of formulation (batch A2)

In the course of IR study, it was found that all the important peaks were present, which is confirmed with the IR of standard drug of itraconazole. The presence of carboxylic acid, alcohol, amide group shows characteristic peaks in formulation. There is no any specific peak, since no any drug interaction in formulation with other excipients. The drug is compatible with other excipients.

Drug- excipient Interaction study by DSC Thermogram:

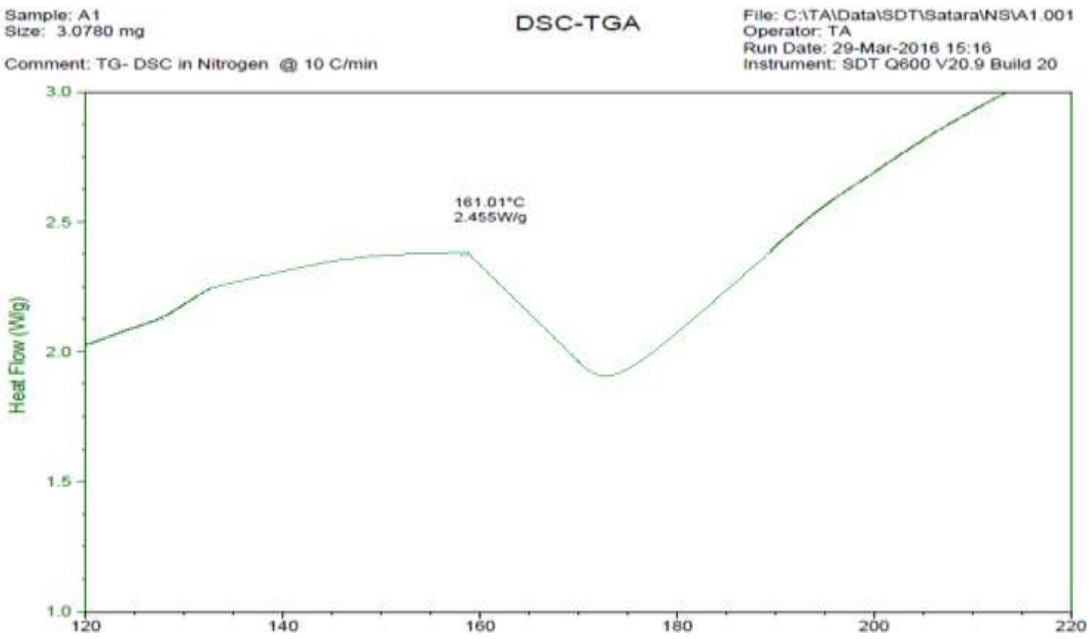

Figure 6: Drug- excipient Interaction study by DSC Thermogram

DSC studies were performed on the individual components and formulations in order to study the interaction between drug and polymer in the formulations to investigate the thermal behavior of formulation mixture.

According to thermo gram, peak was observed at 161.01^{o}C. Pre transition was observed and also broadening of peak was observed because of presence of nitrocellulose. In final formulation alcohol was present for that reason peak was observed at low temperature; because of thermal effect alcohol was evaporated from the sample.

Table 6: Percentage of drug content

Batches	% Drug content
A1	97.66 ±1.52
A2	97.33 ± 1.51
A3	98 ± 2
A4	97 ± 1
A5	97.68 ±1.52
A6	97.66 ±2.81
A7	98.33± 1.52
A8	96.22 ± 1.42
A9	98.33 ± 2.08

(Average of three trials (n=3))

***In- Vitro* Diffusion studies:**

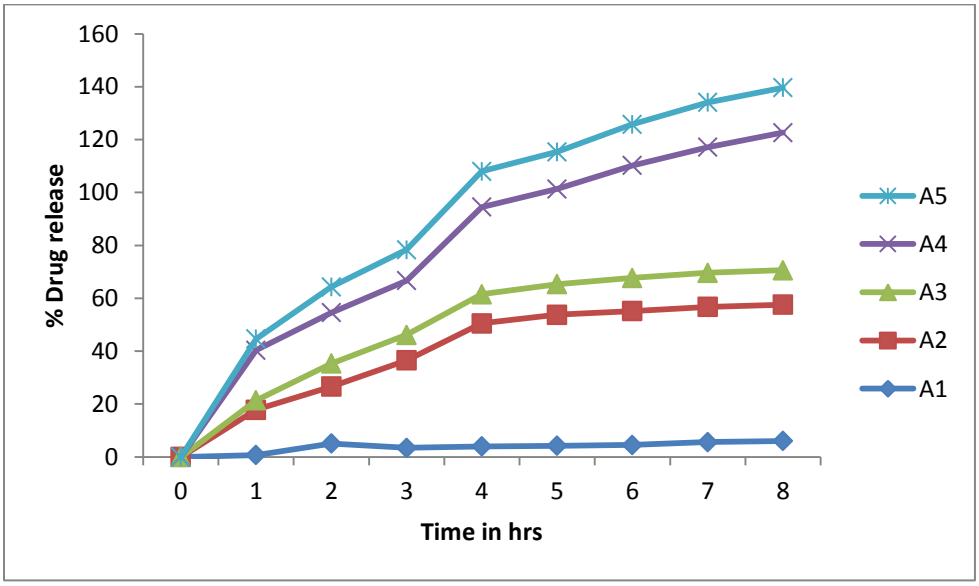

Figure 7: Graph of Cumulative Drug release of Batch A1 to A5

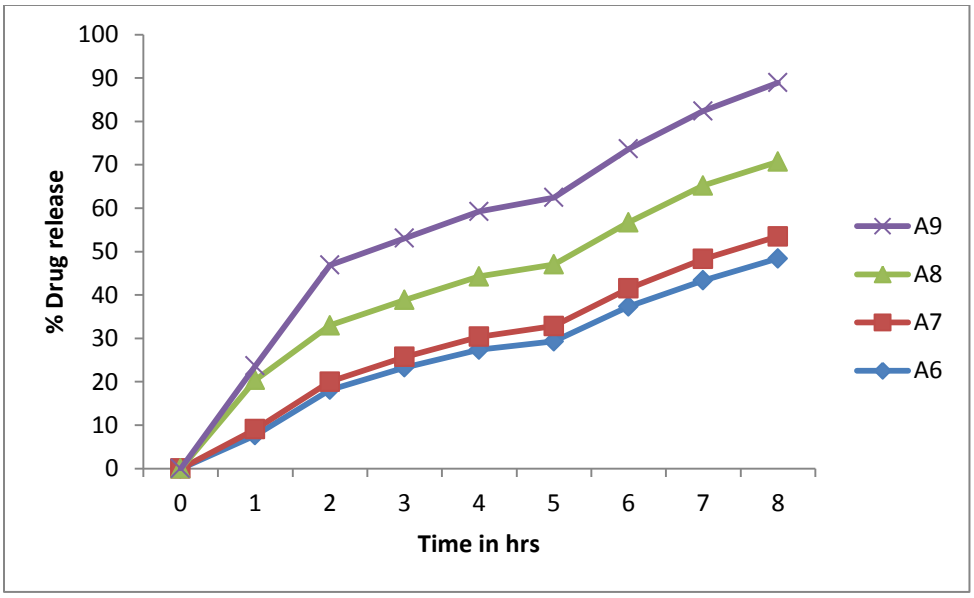

Figure 8: Graph of Cumulative Drug release of Batch A7 to A9

The percentage drug release of A2 and A4 showed better drug release, among the two formulations A2 showed higher drug release rate.

The selected permeation enhancers have varying mechanism in enhancing the permeation of itraconazole. DMSO interacts with the lipid domains of the nail plate by increasing the fluidity or increasing the partitioning of the drug into it. Thioglycolic acid and DMSO is a better penetration enhancer. Formulation with combination permeation enhancers also increases permeation rate of itraconazole through the nail plate.

Stastical Analysis:

A 3^2 full factorial design was selected and the 2 factors were evaluated at 3 levels. The amount of Thioglycolic acid (A) and DMSO (B) were selected as independent variables and the dependent variables were drying time and % of permeability. The data obtained was treated using Stat-Ease Design Expert software.

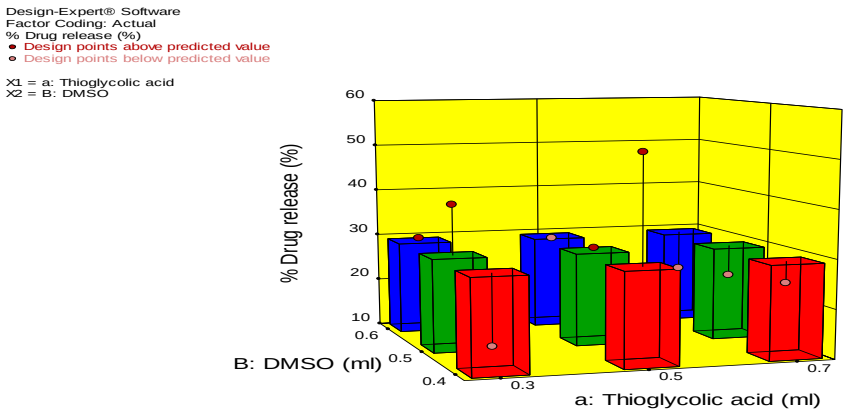

Figure 10: Response 3D surface plot for % permeability

The 3D surface plot shows better drug permeability, when the concentration of thioglycolic acid (X1) and DMSO (X2) used in combination (0.5:0.5 ml).As the other batches shows low permeability of drug as the concentration of permeation enhancer.

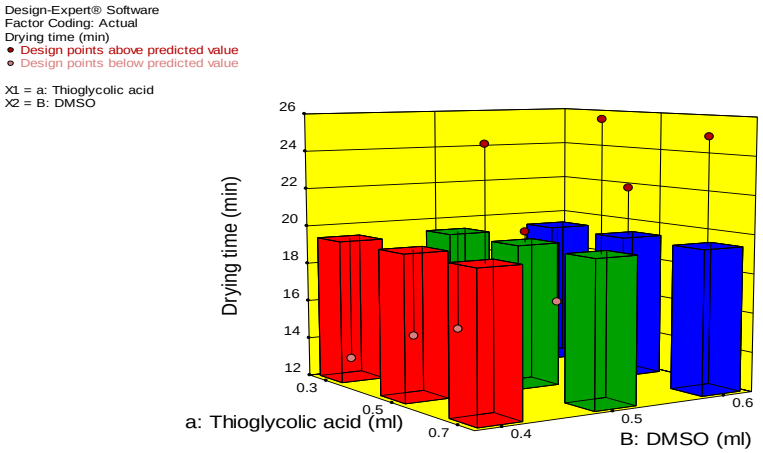

Figure 11: Response 3D surface plot for drying time.

The 3D surface plot shows drying time, when the concentration of thioglycolic acid (X1) and DMSO (X2) used in combination (0.6:0.5 ml). As the concentration of DMSO (X2) decrease the drying time increases.

Table 9: Cumulative Drug release of Batch A1 to A5

Time in hrs.	Percentage drug release				
	A1	A2	A3	A4	A5
0	0	0	0	0	0
1	0.7± 0.1	17.15±0.02	3.53±0.43	18.95±1	4.37±0.12
2	5.07± 0.53	21.54±0.07	8.70±0.36	19.27±1.52	9.7±1.27
3	3.42± 0.01	33.12±0.11	9.62±0.20	20.52±1.62	11.65±0.68
4	3.96± 0.02	46.52±0.58	11.01±0.51	32.98±1.41	13.52±1
5	4.21±0.15	49.53±0.95	11.57±0.21	36.02±1.56	14.02±0.82
6	4.54±0.26	50.61±1.69	12.52±0.30	42.58±1.46	15.52±1.25
7	5.62±0.01	51.09±0.13	12.92±0.42	47.52±1.21	16.92±0.26
8	6.01±0.03	51.58±0.28	13.02±1.52	52.01±0.57	17.01±2

(Average of three trials (n=3))

Table 10: Cumulative Drug release of Batch A6 to A9

Time in hrs.	Percentage drug release			
	A6	A7	A8	A9
0	0	0	0	0
1	7.68±0.01	1.37±0.36	11.37±0.31	3.23±0.75
2	18.13±0.21	1.87±1.76	12.98±0.28	13.92±0.38
3	23.28±1.72	2.46±1.21	13.12±0.75	14.21±0.24
4	27.38±2.7	2.98±2.5	13.92±1.85	14.97±1.46
5	29.79±0.38	3.57±1.28	14.17±0.98	15.41±1.26
6	37.35±0.23	4.16±1	15.21±2.12	16.93±0.83
7	43.36±0.28	4.92±0.62	16.92±1.78	17.21±0.93
8	48.39±1.79	5.12±0.82	17.21±1.29	18.21±1

(Average of three trials (n=3))

Antifungal activity:

Antifungal activity showed result for formulated itraconazole nail lacquer against fungal strain of *Candida albicans.*

Figure 12: Zone of inhibition of A2 and A4 batch formulation

The formulated nail lacquer containing itraconazole showed a zone inhibition 47mm and 48mm against *Candida albicans* respectively. From the result obtained it was concluded that the prepared nail lacquer formulation exhibited promising antifungal activity.

Stability studies:

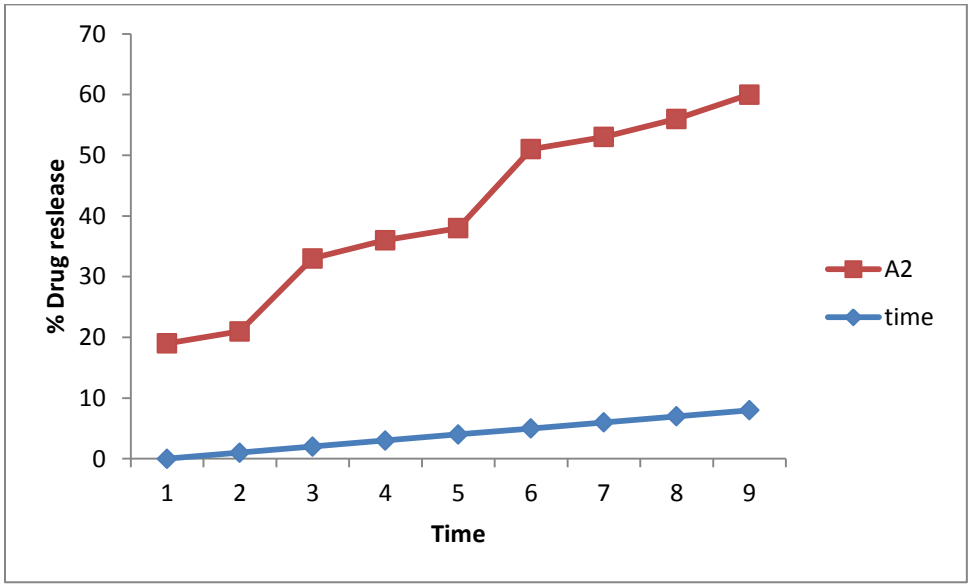

Figure 13: Response of A2 batch after 2 months

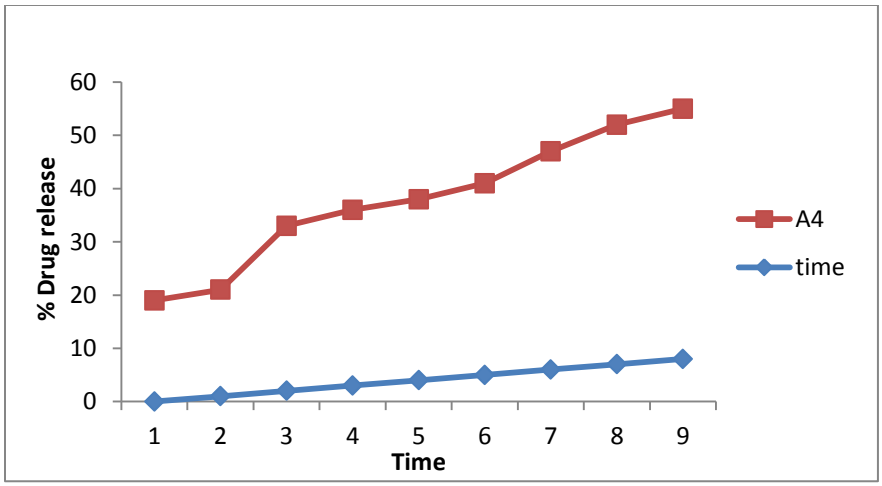

Figure 14: response of A4 batch after 4 months

The stability studies were carried out for A4 at 40± 2°C / 75± 5% RH for 2 month. The results indicated that the nail lacquer does not show any physical change during the study period and drug release was found to be 43.58 at the end of 2 month. This indicates that the nail lacquer is stable at storage conditions.

Table 7: IR interpretation of batch A4

Sr. No	Wavenumber (cm^{-1})	Range	Functional groups associated
1	1642.39	1680-1630	Amides
2	1273.3	1300-1000	Alcohol, ethers, esters, carboxylic acid, unhydride
3	1718.07	1705-1725	Ketones
4	832.59	690-900	Aromatic C-H

Table 8: IR interpretation of batch A2

Sr. No	Wavenumber (cm^{-1})	Range	Functional groups associated
1	2934.42	3000-2850	C-H Alkanes
2	1274.35	1300-1000	Alcohol, ethers, esters, carboxylic acid, unhydride
3	1643.0	1705-1725	Amide

Table 11: Design Summary

Factor	Name	Unit	Type	Coded Level			Actual Level		
				Low	Medium	High	Low	Medium	High
A	Thioglycolic acid	ml	Numerical	-1	0	+1	0.5	1	1.5
B	DMSO	ml	Numerical	-1	0	+1	0.25	0.5	0.75

Table 12: Response Summary

Factor	Name	Unit	Type	Coded Level			Actual Level		
				Low	Medium	High	Low	Medium	High
A	Thioglycolic acid	ml	Numerical	-1	0	+1	0.5	1	1.5
B	DMSO	ml	Numerical	-1	0	+1	0.25	0.5	0.75

Table 13: The responses of all formulations (A1 – A9)

Response	X1	X2	CADD (%)	Drying time(Sec)
A1	-1	-1	6.01	55
A2	-1	0	51.58	92
A3	-1	+1	13.02	77
A4	0	-1	52.01	97
A5	0	0	17.01	58
A6	0	+1	48.39	64
A7	+1	-1	5.12	49
A8	+1	0	17.21	81
A9	+1	+1	18.21	78

Table 14: Results of antifungal activity of batches

Sr.no.	Sample code	Zone of inhibition (mm) against *Candida albicans* of batch A2	Zone of inhibition (mm) against *Candida albicans* of batch A4
1	A-Formulation	47mm	48mm
2	B –Drug	25mm	27mm
3	C-control	22mm	38mm

Table 15: Report of stability studies

Parameters evaluated	Report of A4 batch	Result of A2 batch
Non-volatile content	38.2	41.03
Drying time	72 sec	75 sec
Smoothness of flow	Good	Good
Gloss	Satisfactory	Satisfactory
Water resistance	High	High
Diffusion studies	42.48%	51.58%

Table 16: Stability Analysis of A2 batch

Conditions for stability	Time interval (in months)	Formulation (A2 batch) Drying time(seconds)	Permeability (%)	Appearance
At 4-5^{o}C	0	72	51.74±0.01	Transparent, no any colour change
	1	72	51.72±0.23	Transparent, no any colour change
	2	74	52.34±0.24	Transparent, no any colour change
At 40^{o}	0	71	51.74±0.01	Transparent, no any colour change
±2^{o}c/75%	1	75	52.72±0.23	Transparent, no any colour change
±5%RH	2	76	52.31±0.24	Transparent, no any colour change

Table 17: Stability study of A2 batch after 2 months

Conditions for stability	Time interval (months)	Formulation batch (A4 Batch) Drying time(in sec)	Permeability (%)	Appearance
At 4-5^{o}C	0	67	47.52	Transparent, no any colour change
	1	72	47.46	Transparent, no any colour change
	2	68	46.89	Transparent, no any colour change
At 40^{O}c 2^{o}c	0	65	47.52	Transparent, no any colour change
/ 75% 5%	1	68	47.67	Transparent, no any colour change
RH	2	71	48.34	Transparent, no any colour change

CONCLUSION

The main objective behind formulating the antifungal nail lacquer is to check the suitability in delivering the drug to the targeted area. As area between nail and skin generally affected is hard to reach through topical medication. The aim behind formulating the antifungal nail lacquer could serve a good option in delivering drug to those hard to reach area. The nail lacquer formulation were prepared using itraconazole using simple mixing method. Which has low permeability through its topical drug delivery? The permeability of drug enhanced by using the permeation enhancer (thioglycolic acid and DMSO).As the permeation enhancers used in combination the drug permeability enhanced and the drying time increased. Since the drug retained for longer period of time for its permeability. The prepared medicated nail lacquer evaluated for various parameters such as non-volatile content, drying time, smoothness of flow, gloss, water resistance, diffusion studies that shows satisfactory results.

The permeability studies show the better permeation of drug at those hard to reach area. These nail lacquer solutions applied to the site, retained as film, that ensure prolong delivery of drug. The prepared nail lacquer not only used for the nail infections, but also it can be used in the cosmetics for the beautification of nails.

Optimized batch of nail lacquer A2 and A4 showed high permeability i.e. 51.58% and 42.48% by using the permeation enhancers (thioglycolic acid and DMSO) in different concentrations

((0.3,0.4ml) and (0.5,0.4ml)). *In vitro* drug diffusion of nail lacquer formulation showed required release in desired period of time.

It can be concluded that, the nail lacquer formulation containing itraconazole is patient friendly and it is effective dosage form for treating fungal nail infection.

Ex-vivo on nail plates of human volunteers and clinical studies to check its therapeutic effectiveness can be done. In future the scale up studies can be performed to find the various lacunae during large scale manufacturing of nail lacquer. The other drug candidates can be explored to treat the different nail infections.

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REFERENCES

1. Kajale R. P, More S. R, Takle S. J, Jadhav A. An emphasis on nail infection. Indo American Journal of Pharmaceutical Research. 2015:5(12);3765-75.
2. Patel R.P, Naik S.A, Patel N.A, Suthar A.M. Drug delivery across human nail. International Journal of Current Pharmaceutical research 2009:1(1);1-7.
3. Colin Dollery, Itraconazole: Therapeutic drugs 2nd ed. Churchillivingstone publisher; 1999 P 120- 125.
4. Raymond C R, Paul J S, Marian E Q. Handbook of pharmaceutical excipients, 6th ed. Pharmaceutical press publisher: 2009.
5. Rowe R C, Sheskey P J, Weller P J. Hand book of pharmaceutical excipient. 4th ed. U K, Science and Practice Publishers, 2003; 101, 271-273, 959.
6. Chatwal G R, Anand S K. Instrumentation Methods of chemical Analysis. Himalaya publishing house. Edited by- Arora H, Anand A. 5th edition: 2.747-2.752.
7. Kalsi P S. Spectroscopy of organic compounds. New age international publisher 4th edition: 59-163.
8. Desai A.S, Shripati D. Formulation and In vitro Characterisation of nail lacquer containing flucanazole for preungal Drug Delivery system, International and Journal of pharmaceutical Research Bioscience 2014:3(2); 200-14.
9. Chandra. R, Kumar S, Aggarwal A. Evaluation of nail lacquer. Indo Global Journal of Pharmaceutical Sciences. 2012; 2(4):379-382.

10. Indian pharmacopeia (1996). Government of India. Ministry of health and family welfare (4th ed).Delhi; The controller of publication.
11. ICH Q 1 (A)- Stability testing for product, February 2003.
12. Parashar B, Yadav V, Maurya B, Sharma L. Natural therapy of fungal nail diseases: Review.www.the pharmajournal.com 2014:1(4); 31-47.
13. Robert D.T., Taylor W.D., Boyle J. Guidelines for treatment of onychomycosis, British Journal of Dermatology. 2003:148; 402-10.
14. Patil S.P, Sangita V, Basrur P.R, Katedeshmukh R.G. Review on nail (transungual) Drug delivery. European Journal of Pharmaceutical and Medical Research. 2015:2(2); 551-71.
15. Suryavanshi K.A, Basrur .P.R, Katedeshmukh R.G. Review on nail (Transungal) Drug Delivery System. American Journal of Pharmatech Research. 2012:2(5);223-44.
16. Flowerlet M, Bindumol KC, Jimsha P, Rolicin PP, Vincy V. Understanding our natural nail antifungal agents. International Journal of Pharmacy and Pharmaceutical Science .2014:6(2);918-22.
17. Kumar R.J, Selvadurai M, Subramani .P. Antifungal Agents: New Approach For Novel Delivery Systems, Journal of Pharmaceutical Science and Research. 2014:6(5);229-35.
18. Khanna S, Bajaj R, Khurana B, Srivastava K. Pharmacotherapeutic Principles of Ungual Drug Delivery System. International Journal of Drug Development and Research. 2012:4(3);9-18.
19. Mittal. A, Maiti. A, Jha. K. K. Formulation, Evaluation and Optimization using full factorial design based Diclofenac Potassium Sustained Release Micropellets. www.thepharmajournal.com. 2013:2(9);1-9.
20. Shireesh R.K, Shekar B.C, Vishnu P, Prasad M V. Ungual Drug Delivery System of Ketoconazole nail lacquer. International Journal of Applied Pharmaceutics. 2010:2(4); 16-19.
21. Azharuddin M, Bhatt S, Shabaraya A. R, Prasad N. G. Zilu, Preparation and In-vitro evaluation of antifungal nail lacquer ,International journal of universal pharmacy and bioscience, 2013:2(4);78-85.
22. Bhimavarapu R, Karuna P.C, Raviteja G, Karunkiran P, Mehuragavendra Y, Sundaramma S. Itraconazole loaded microsponges-A novel Carrier system. International Journal of Inventions in pharmaceutical Science. 2015:3(1); 953-57.
23. Puri.D, Trivedi S. Formulation and evaluation of Terbinafine Hydrochloride As a Nail Lacquer. Acta Velit.2014:1(1);25-33.

24. Bhapkar.P.H ,Puttewar.T.Y,Patil.R.Y, Nail Lacquer in Nail Diseases,IOSR Journal of Pharmacy,2013:3(9);24-48.
25. Debijit.B, Gopinath H, Pragati.B. K, Duraivel S, Aravind G, and Sampatkumar K.P. Nail Drug Delivery System A Novel Approaches For Drug Delivery System. The Pharma Innovation. 2013:1(11);3-14.
26. Bristow-I.R. The Effectiveness of lasers in the treatment of onychomycosis: a systematic review. Bristow Journal of Foot and Ankle Research 2014:7(34); 3-10.
27. Elezovic. A, Hadzovic S. Simple, Inexpensive and ecologically friendly derivative spectrophotometric Fluconazole Assay From Nail Lacquer Formulation. American Journal of Analytical chemistry, 2012:2; 109-15.
28. Dileep P, Vijaya V. S, Rao U. M. Formulation and Evaluation of Itraconazole Ethosomal Gel. International Journal of Pharmacy Review and Research.2015:5(2);85-94.
29. Poornim G, Sandhyarani K, Brahmaiah B, Padmaja.K . Nail Drug Delivery- A novel Approaches for Drug Delivery System. International Journal Of Advanced Pharmaceutics.2013:3(2); 102-9.