IN-VITRO EVALUATION OF AN ANTI-FUNGAL NAIL LACQUER CONTAINING MICONAZOLE NITRATE

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ABSTRACT

The primary goal is the formulation and evaluation of an anti-fungal nail lacquer that contains miconazole nitrate and is intended to treat the skin condition known as onychomycosis, which is brought on by pathogens such as candida, dermatophytes, and non-dermatophytes. Nail lacquer is also useful for enhancing therapeutic effectiveness and ensuring patient compliance. It can be made by simply combining non-volatile ingredients and achieving the desired gloss, flow, and smoothness. Humans use nail lacquer on their fingernails and toenails. In order to enhance beauty, gloss, and colour, it is important to protect the nail and nail plate. In order to increase the topical absorption of the drug over the nail, nail lacquer is mostly utilised for medications with poor oral bioavailability. This preparation uses a variety of ingredients, including 2 hydroxy propyl beta cyclodextrin, ethyl cellulose, nitrocellulose, and propylene glycol, to construct the medicine and provide the best possible release.

Keyword: Fungal Infection, Nail Lacquer, Onychomycosis.
DOI Number: 10.14704/nq.2022.20.11.NQ66272 **NeuroQuantology 2022; 20(11): 2699-2714**

INTRODUCTION

The nail has an edgy design. The nail plate is what allows drugs to pass through it. When something is tough enough, it becomes difficult to penetrate; just a small amount of topical medication can get through. As a result, the therapeutic concentration is not effective. Reduced luminescence may make the nail plate appear odd. It involves the nail bed, reduces blood flow, or has chemical or physical characteristics of the nail bed. Various diseases end up developing as a result. [1] By using a nail drug delivery system to achieve the desired therapeutic concentration of a medicine, various disorders can be healed. Although human nails do not serve a cosmetic purpose, they can be thought of as a potential medicine delivery system. delivery, particularly in cases of psoriasis or onychomycosis of the nails. These nail conditions are very common among people, especially the elderly and those with weakened immune systems. [2] Topical therapies are constrained by the poor rate of penetration through the nail plate, whereas oral therapies are accompanied by systemic side effects and medication interactions. The administered active medicine must penetrate the thick, keratinized nail plate and reach deeper layers, the nail bed and the nail matrix, in order to successfully treat nail disease. Studies on human skin have clarified its structure, functions, and permeability for some substances, but nothing is known about the nail, its keratin content, or how skin derives from the nail. In order to treat not just external nail illnesses but also potential systemic circulation and nearby target locations, it is important to have a better understanding of the physicochemical factors that affect medication absorption through the nail plate. Because they can't provide enough antifungal medication to the target locations to completely remove the protection, current topical therapies have a limited therapeutic impact. This may be because they can't sufficiently penetrate the nail plate. It is challenging to analyse the drug's permeation as well. To minimise the negative effects of systemic medication for nail illnesses including onychomycosis and, to a lesser extent, nail psoriasis, improve patient compliance, and lower treatment costs, topical therapy is preferred. However, systemic therapy is the basis of treatment because the nail plate does not readily absorb medications when administered topically. Fungal medication penetration needs to be improved for topical therapy to be successful [3]. This can be accomplished by physically or chemically damaging the nail plate. Alternatively, drug permeation into the intact nail plate may be encouraged, for example, by iontophoresis or by formulating the drug within a

eISSN 1303-5150

vehicle which enables high drug partition out of the vehicle and into the nail plate. The physical techniques (manual and electrical nail abrasion, acid etching, ablation by lasers, microporation, application of low-frequency ultrasound and electric currents) and chemicals (thiols, sulphites, hydrogen peroxide, urea, water, enzymes) that have shown fungal enhancer activity. The human nail can develop a number of diseases, including as psoriasis, paronychia, and infections brought on by bacteria, viruses, or fungus. These cause psychological tension and self-consciousness even though they are rarely life-threatening [4]. Onychomycoses, a type of fungal infection, are thought to be the cause of 50% of all issues and may be as common as 27% in Europe and 10% in the US. There are numerous different treatment plans, but the most popular one involves taking antifungal medications orally, including terbinafine or itraconazole. The development of newer, more efficient topical products and regimens was made possible by experimental techniques for the investigation of the penetration and distribution of chemicals into and through the nail plate, which showed that it is possible to deliver drugs to the nail after topical application. A novel ultrasoundmediated drug delivery system has been developed for treatment of a nail fungal disorder (onychomycosis) by improving delivery to the nail bed using ultrasound to increase the permeability of the nail. The nails are composed of flat, horny scales which form protective covering for the distal of the finger & toes [5, 6, 7].

MATERIALS AND METHODS

MATERIALS AND INSTRUMENTS USED

Miconazole nitrate (Yarrow, Chemicals, Mumbai), HP-β-CD (Yarrow Chemicals, Mumbai), Ethyl cellulose (Kemphasol, Popatwadi, Mumbai), Nitro cellulose (Kemphasol, Popatwadi, Mumbai), Propylene glycol (Laboratory grade, Otto kemi, Mumbai), Salicylic acid (Laboratory grade, Nice chemicals Pvt. Ltd. Cochin), Ethyl alcohol (Laboratory grade, Jiangsu Huaxi International Trade Co. Ltd., China), Sodium hydroxide (Laboratory grade, Nice chemicals Pvt. Ltd. Cochin), Potassium dihydrogen phosphate (Laboratory grade, Nice chemicals Pvt. Ltd. Cochin). Electronic weighting balance (Shimadzu Corporation, Japan), FT-IR (Perkin Elmor, USA), Double beam UV spectrometer (Shimadzu Corporation, Japan), Franz diffusion cell (Murthys, Hyderbad), Tensile strength apparatus (Dept. of Pharmaceutics, APSC), Screw gauge (Technico, Delhi).

PREFORMULATIONS STUDIES

Recognition of Drug

A) Study of solubility

Saturated solubility of Miconazole nitrate was made by applying 10 ml of distilled water/ethanol/acetone in 25 ml volumetric flasks in thrice. Precaution was taken so that the drug dosage form stay in medium in spare. Then by using mechanical shaker, the flasks were shaken for 48 hours. The test sampling was done on 24^{th}& 48^{th} hour. The test sample is withdraw (1 ml after filtration) was soluble with suited medium and analyzed by using UV spectrophotometer at 223 nm.

B) Determination of the melting point

Melting point of drug determined by excellent measurement by fetching a few amount of drug in a capillary tube certain at once last and was attached in Thiel's melting point setup and temperature range at that the drug melted was presented. Mean of one of thrice readings was written.

C) λmax determination

100 mg of pure Miconazole nitrate was interpreted in a volumetric flask and soluble in a small few amount of phosphate buffer pH of 7.4 and volume made up to 100ml. 1ml of the trying firstly of dilution was taken and some diluted to 100ml. The trying test firstly solution scanned for excellent absorbance in double beam UV-Visible spectrophotometer in between the range of 400-200 nm against phosphate buffer pH 7.4 as the clean. Thrice reading were taken and mean was determined.

ANALYTICAL METHODS

A) Phosphate buffer solution preparation

0.2M Sodium hydroxide solution preparation

8gm of the sodium hydroxide was soluble in needful quantity of distilled H2O in a 1000ml volumetric medium and volume made up to 1000ml with distilled water.

0.2M potassium dihydrogen phosphate solution preparation –

27.218gm of potassium dihydrogen orthophosphate was soluble in needful quantity of distilled H2O in a 1000ml volumetric medium and volume was made up to 1000ml with distilled H2O.

The pH of phosphate buffer solution preparation

50ml of potassium dihydrogen phosphate solution was taken in a 200ml volumetric flask and 39.1ml of 0.2M sodium hydroxide solution was mixed and made up to 200ml with distilled H20.

B) Standard stock solution & Calibration curve of Miconazole nitrate preparation

Miconazole nitrate 100mg pure drug was right weighed and transfer into a 100ml volumetric flask of medium. And the volume was made up to 100ml with PBS of pH 7.4, to come into ownership standard stock solution of 100mcg/ml concentration. According above solution of 2ml, 4ml, 6ml, 8ml, 10ml, was pipetted out into other 100ml volumetric flask and made up to 100ml with PSB of pH 7.4 come into

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ownership a concentration range of 20µg/ml, 40µg/ml, 80µg/ml, and 100µg/ml solution. The analyzed of solution at 223nm by using UV-Visible spectrophotometer. The concentration versus absorbance was plotted on the graph. Drug constitutes assessment and diffusion presented were aim on this calibration curve.

Drug-polymer compatibility determine

Pure drug FT-IR spectral analysis and polymer were portaged out singly and as composition. The compatibility between Miconazole nitrate, nitrocellulose, 2-HP-β-CD, propylene glycol and made development were carried out in the ratio 1:1. The test was located FT-IR window after mixing and triturating with potassium bromide.

Table.1-Drug-Polymer compatibility study

Composition	Ratio	250 C ±2 /60^0 CRH	40^0 C ±2 /75^{0}C RH
Miconazole nitrate	100mg	6 Months	1 Month
Nitrocellulose	100mg	6 Months	1 Month
HP-β-CD	100mg	6 Months	1 Month
Propylene glycol	100mg	6 Months	1 Month
Miconazole + nitrocellulose	1:1	6 Months	1 Month
Miconazole + HP- β-CD	1:1	6 Months	1 Month
Final Formulation	NA	6 Months	1 Month

FORMULATIONS STUDIES

Preparation of nail lacquer of Miconazole nitrate

A) Making of Nitrocellulose

Approximate 5gms of cellulose base (cotton) is mixed to 50ml concentrated sulfuric acid and 25ml 70% nitric acid mixture and chilled to 5-10 ^{0}C to give cellulose nitrate. Then cotton was separated and washed in chilled water and with $NaHCO_3$ Solution separated all acid remain. It was then low at dried at room temperature.

B) Optimization of Nitrocellulose film former

Table.2-Optimization of nitrocellulose film former

Formulation Code	Nitrocellulose (% w/v)	Plasticizers (% w/v)		Ethanol (ml)
		PG	Glycerin	
NF1	3	10	....	10
NF2	5	10	....	10
NF3	7	10	....	10
NF4	9	10	....	10
NF5	3	....	10	10
NF6	5	....	10	10
NF7	7	....	10	10
NF8	9	....	10	10

4 different concentrations of nitrocellulose, 2%, 4%, 6%m 8%, were made applying 2 different plasticizers, Propylene glycol and glycerin at 10% concentration as per Table No. 2 **.**The optimal concentration for film formation was characterized by great determination by rating the thickness, tensile power, folding stress and H2O opposition.

Evaluation

a) Film thickness

The thickness of the flick was determined by applying screw gauge with a minimum count of 0.01 mm at many points of the films. The thickness was

b) Folding Endurance

Folding endurance of the films was measured by repeat foldaway a little strip of the film (approximately 2x2 cm) at the same site till it brittle. The numerous of times film could be crimped at the same site, without brittle gives the factor of folding endurance.

FORMULATIONS STUDIES

Preparation of nail lacquer of Miconazole nitrate

A) Preparation of Nitrocellulose

Around 5gms of cellulose base (cotton) is mixed to 50ml concentrated sulfuric acid and 25ml 70% nitric acid mixture and chilled to 5-10 ^{0}C to give cellulose nitrate. Then cotton was separated and washed in chilled H2O and with $NaHCO_3$ Solution to separate all acid remain. It was then easily slow dried at room temperature.

t=thickness of sample in cm.

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b)-Water resistance

This is determine of the resistance to the aqueous permeability of the layer. This was by applying a continuous layer on a plane and plunging it in water. This weight before and after submergence was written and maximize in weight was calculated. Larger the maximize in weight lesser the water resistance.

Development of nail lacquer-

TABLE.3-FORMULATION COMPOSITION

The Formulation was done according to formula shown**.** Miconazole nitrate and Nitrocellulose was solubilised in Ethyl alcohol in the important substance used a magnetic stirrer at an various speed. To clear the solution important substance of 2-HP-β-CD, Salicylic acid, and propylene glycol were mixed and volume to 100ml. The prepared nail lacquer was Trans change to a narrow plastic screw capped glass bottle.

Ingredients (%)	F0	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Miconazole nitrate	3	2	3	3	3	3	3	3	3	3	3	3
Nitrocellulose	7	7	7	7	7	7	7	7	7	7	7	7
Salicylic Acid		6	11	16	21	16	16	16	16	16	16	16
2-H-β-CD	...	...	...	...	...	5	7.9	11	11	11	11	11
Ethyl cellulose	...	...	...	...	...	...	...	...	0.26	0.51	0.79	1.09
Propylene Glycol	11	11	11	11	11	11	11	11	11	11	11	11
Ethanol q.s	100	100	100	100	100	100	100	100	100	100	100	100

EVALUATION OF NAIL LACQUER

A) Nonvolatile content

10ml of preparation was take in a petri dish and firstly weighed were taken. This dish was put in the oven at 105^0C for 1hr, the petri dish was removed, cooled and weighed. This separated in weights was taken. Mean of one of three cycle readings was reported.

B) Drying time-A layer of formulation was used on a petri dish with the using by the brush.. The time for make a dry-to-hard layer was noted use by stop watch.

C) Smoothness to flow

The preparation was dip from a heighted of 1.5 inches into a glass plate and dispersed on a glass plate and made to wave vertically and see obtaining for smoothness of layer.

D) Gloss

Development of nail lacquer was used on the nail and gloss needful and done with marketed cosmetic nail lacquer.

FORMULATIONS STUDIES

1 Development of nail lacquer of Miconazole nitrate

E) Viscosity using the brook field viscometer.

F) Adhesion

There are neither to amount of evaluation tools resultant to use the medicinal nail lacquer at this time of duration. The instruments is used of chemical balance applied in the general laboratory as showed. One pan of the balance was transfer with two stainless steel plates. In between the plates a film of 4 cm^2 was made and adhered. The poise of the balance was adjusted by mixing a weight to the right pan of balance. The force needful to pull away the plates determined and compared with a commercial cosmetic nail lacquer test sample.

G) Drug content appraisal-

Nail lacquer equivalent to 200mg was soluble in 50 ml phosphate buffer solution of pH 7.4. Then the solution was supersonic for 15 mints. Resultant solution was filtered, made up to 100 ml with phaphate buffer solution of pH 7.4. From the above solution carried at 10ml and made up to 100ml with PBS of pH 7.4. Then the diluted solution was assesssment spectrophotometrically at wavelength of 223 nm and determined the drug constiuents.

H) Diffusion studies across artificial membrane

Diffusion studies were tested by Franz cell applying artificial membrane (cellophane) of 0.8μm. The membrane was loaded for 24hrs in solvent system and the solvent fill the receptor compartment.

Nail lacquer equivalent to 200mg was used evenly on the surface of the membrane.

The made membrane was assembled on the cell carefully to avoid entrapment of air bubbles in the membrane. The all weldment was maintained at 37^0C, and the speed of stirrings was kept constant for 20hrs. The 5ml aliquot of drug sample was taken at time intervals of 2hr, 4hr, 8hr, 10hr, 12hr, 16hr, and 20hrs and was replaced by the fresh solvent. Samples were analyzed by double-beam UV spectrophotometer as per method mentioned in drug content appraisal. Each experiment was recurrent thrice.

I) In vitro permeation studies

Hooves from freshly slaughter cattle, free of adhering tending to attach and cartilaginous tissue, were loaded in distilled water for 24hrs. Membranes of approximate 1mm thickness were cut form the distal part of hooves. In vitro permeation studies were tested by using from Franz diffusion cell, the hoof membrane was situated by paying attention on the surface of the nail membrane. The targeted receptor compartment was filled with solvent phosphate buffer

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solution of pH 7.4, and the all weldment was maintained at 37^{0}C with constant mixing for 48hrs. The 5ml factor of number of drug sample was taken after a time intervals of 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48hrs. transfered by the fresh solvent. The drug analysis was done by using double-beam UV spectrophotometer at 223nm.

J) Determination of antimicrobial activity

Candida albicans were wages for testing antifungal act by the cup-plate method. The culture was take up on sobouraud's agar slants. 20ml of melted sobouraud's agar medium was confirm 72hrs. Old 0.2 ml suspension of Candida albicans in the Petri dish and allowed to standard by conformity undisturbed for 15 mints.

The cups (10mm diameter) were slugged in the Petri dish and filled with 0.05 ml of a solution of the sample. The plates were taken for diffusion at 40^{0}C for 1hr, and followed by incubation at 30^{0}C for 48 hrs. After done the incubation time the zone of suppression in millimeters were determined. On with test solution in every petri dish one cup was filled up with solvent, which play as control. The zone of suppression was noted and compared with control.

K) Stability study

Stability studies of nail lacquers were according ICH guidelines. Test samples were at temperature of 25±2 ^{0}C/60 ±5% RH for 6 months and 40 ± 2^{0}C/75 ± 5% RH for 1 month. Then the samples were analyzed for non-volatile content, drying time, gloss, smooth of flow, drug content and diffusion across artificial memb

RESULT AND DISCUSSIONS

Results for Analytical Study

Scanning of drug

Pure Miconazole nitrate sample was scanned using phosphate buffer solution (PBS) of pH 7.4 between 200nm to 400nm using UV visible spectrophotometer. The tallest peak of Miconazole nitrate was obtained at 223nm **(Figure 12)** and thus the λ_{max} of Miconazole nitrate was at 223nm and was used some spectrophotometric evaluations during the investigation.

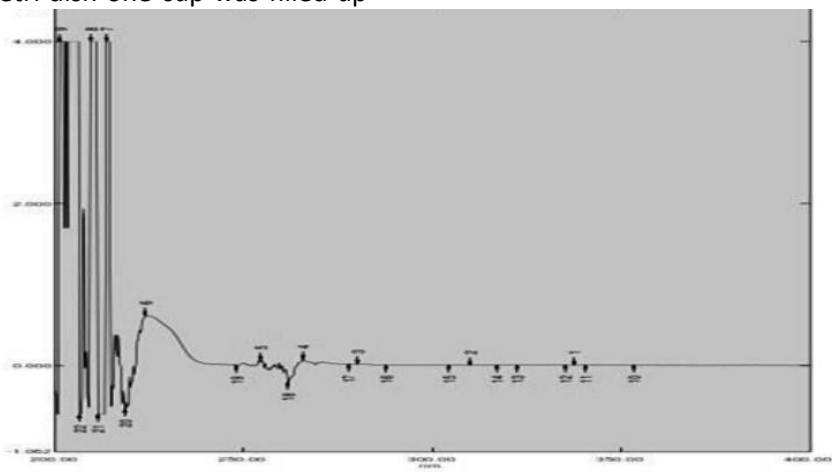

Figure 1: UV spectrum of Mixonazole nitrate in phosphate buffer solution of pH 7.4

Standard curve for Miconazole nitrate in phsphate buffer of pH 7.4

Standard solutions of Miconazole nitrate in various concentrations (Table below) were made applying PBS pH 7.4 and their absorption was determined at 223nm. Drug concentration Vs. absorbance was plotted**.**

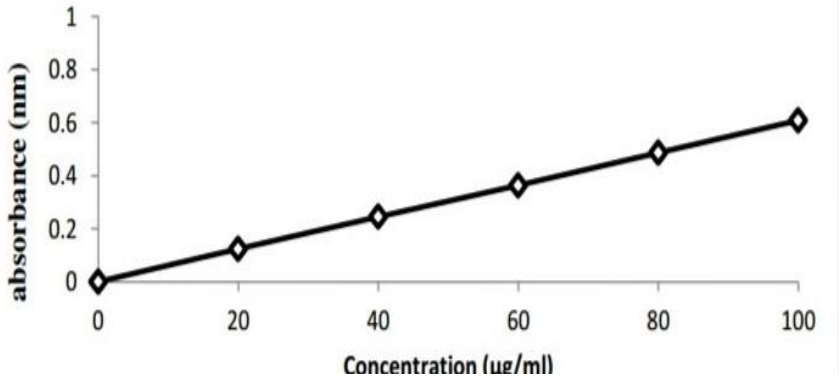

Figure 2: Calibration curve of Miconazole nitrate in phosphate buffer solution pH 7.4

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2 PREFORMULATIONS STUDIES

1 Solubility studies of Miconazole nitrate

The result of solubility studies of pure Miconazole nitrate are given below:

Table No. 4: Solubility studies of Miconazole nitrate

Solvents	Solubility (mg/ml)
Ethanol	0.78
Water	0.03
Acetone	0.36

From the data, solubility profile of Miconazole nitrate was insoluble in water, soluble in ethanol and acetone.

3. Melting point determination

The melting point was found to be 161^0C $\pm$ 0.577 and as per the IP 2007 melting point of Miconazole nitrate was within the range of 159-160 ^{0}C.

4 Drug excipient compatibility study

All the reference IR peaks of the pure drug Miconazole nitrate were also present in the spectra of mixture of drug-polymer and drug-permeation enhancer-excipients as mentioned in the above Table No. 10.

So FTIR study showed that there is no interaction between drug and permeation enhancer. So the drug and permeation enhancer are compatible. The IR spectrums were given in the Figure below.

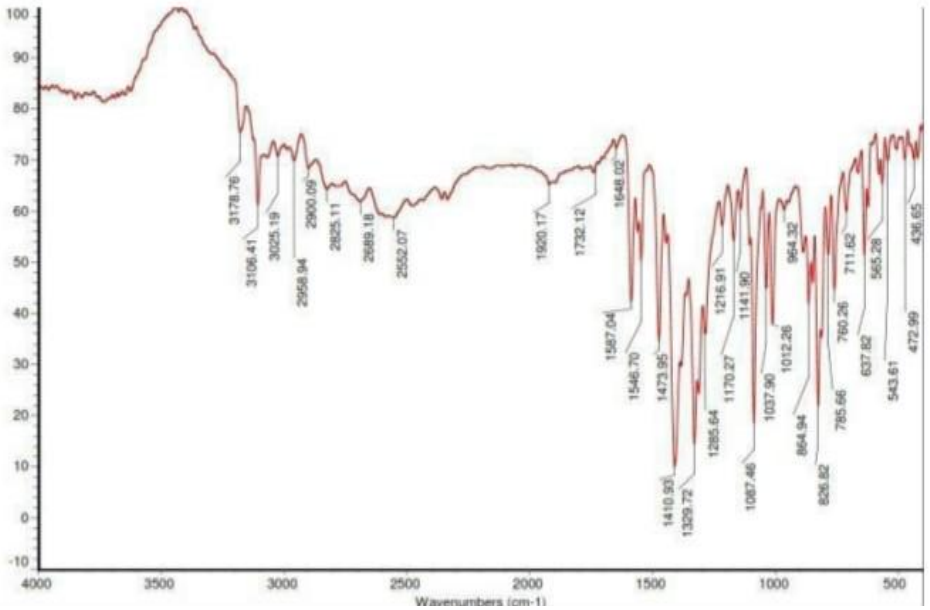

Figure 3: IR spectra of miconazole nitrate

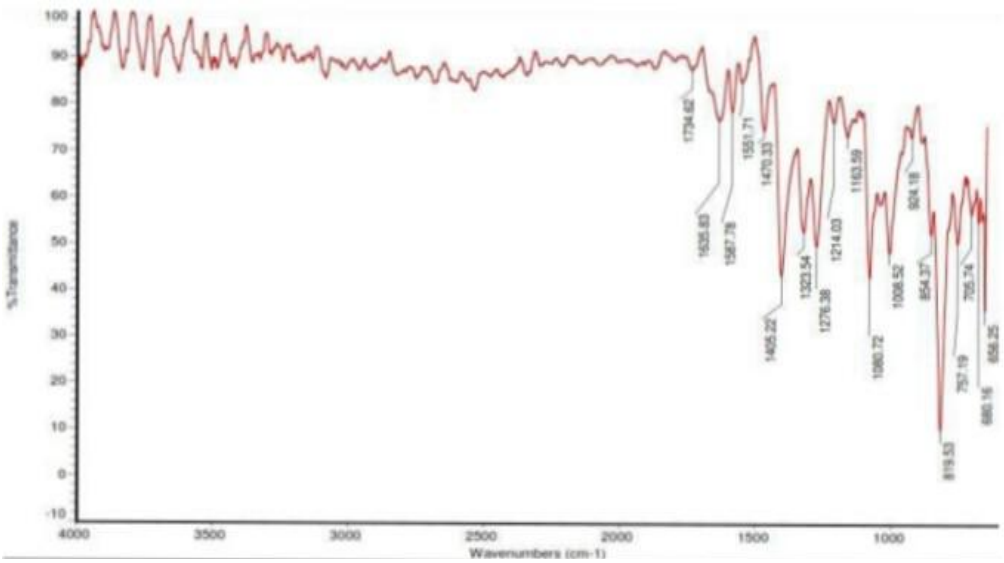

Figure 4: IR spectra of nitrocellulose

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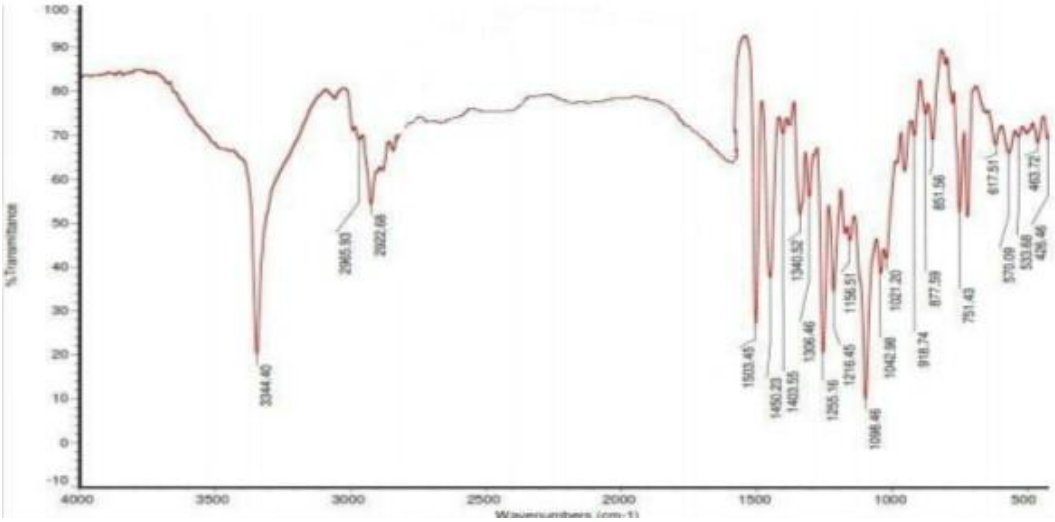

Figure 5: IR spectra of ethyl cellulose

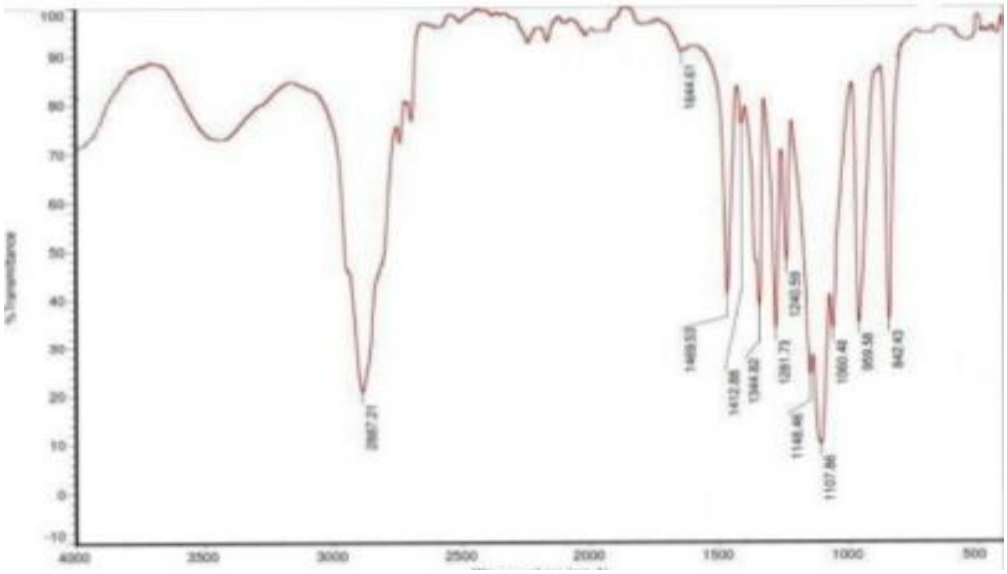

Figure 6: IR Spectra of the beta hydroxyl propyl cellulose

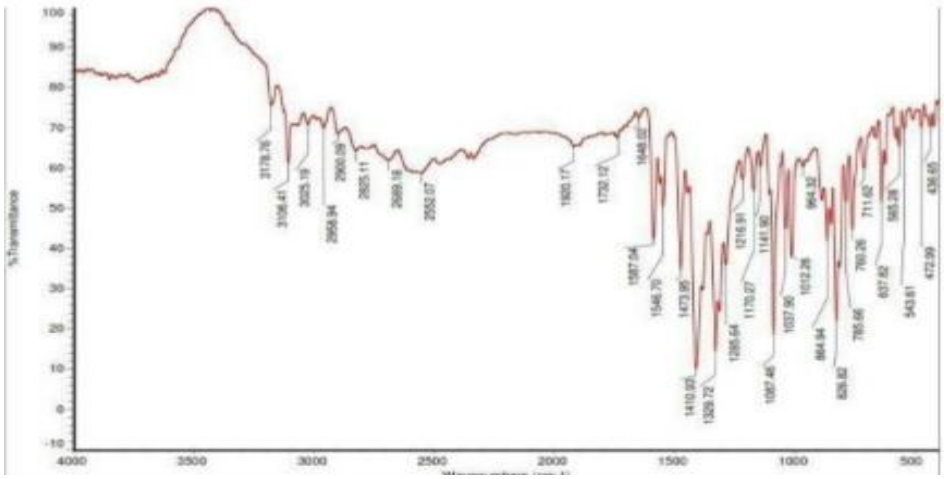

Figure 7: IR Spectra of Miconazole nitrate and nitrocellulose

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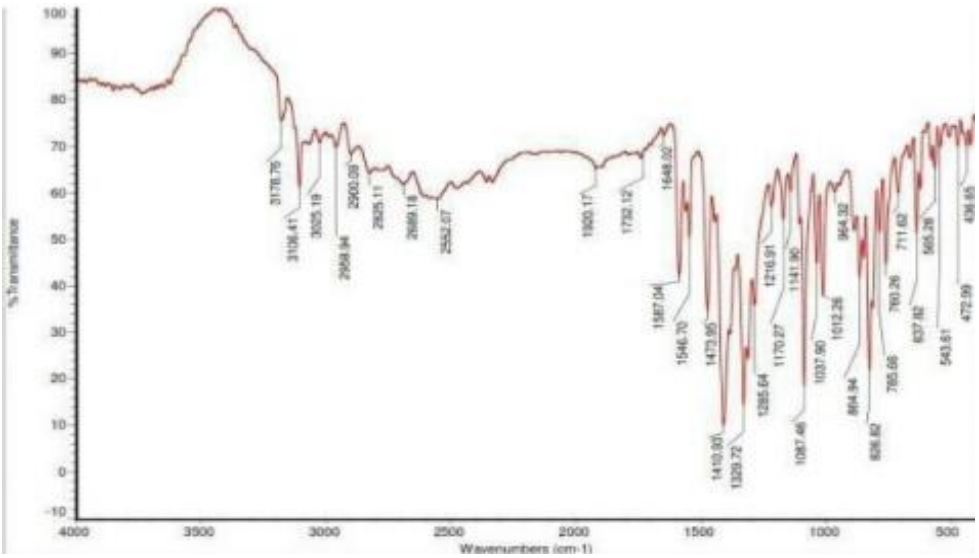

Figure 8: IR Spectra of Miconazole nitrate and beta hydroxyl propyl cellulose

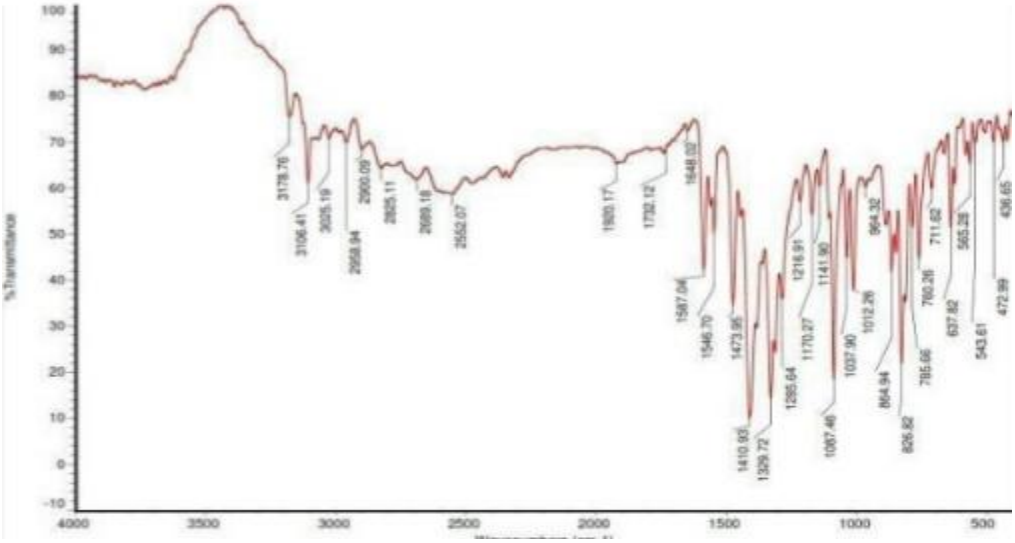

Figure 9: IR Spectra of Miconazole nitrate optimized Nail lacquer

After spectral comparison it was confirmed that not compatibility reaction took place between drug and additives, as all main properties IR peaks of Miconazole nitrate are present in the physical mixture with individual additives and also in the final optimized formulation, F11. All the additives peaks were obtained to be entire indicating nice compatibility.

6.4 Formulation development of Nail Lacquer

The aim of the present study was to furnish a preparation for conquer fungal developed on toe nails or finger nails so that the looks of the nails are valuable. Preparation consists a film former nitrocellulose, permeation enhancer such as 2-H-β-CD, keratolytic agent like salicylic acid and an antifungal agent (Miconazole nitrate) and ethanol as solvent. Preparation is made by simple mixing method.

5 Optimization of nitrocellulose film former

Various concentration of film forming polymers were applied for film formation and then applied for optimization of film. Various concentrations were tried between 2-8%. From the conclusion, it was obtained that by maximizing the concentration polymer up to 6%, thickness and strength of film was coveted. While maximizing concentration more than 6%, sticky films were generated. Thus, 6% concentration of polymer was needful for some obtained of plasticizer. Plasticizer tried were Glycerin and Propylene glycol in 10% concentration each. Glycerin showed more sticky film which was unable to detach from surface. Thus, 6% nitrocellulose and 10% propylene glycol, due to its excellent film forming nature was choose for some optimization research.

A) Thickness (μm)

Unvarying thickness bespeak the unvarying of the preparation because of that suitableness of the executed procedure. Thickness of all the films determined by applying a micrometer screw gauge. Obtained result presented that thickness of all preparation varied from 55 to 59 μm.

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The determined values were shown in the Table No. 16**.** Data for film thickness was duplicate within the coveted range of thickness identified through review of literatures for films.

B) Folding endurance

Folding endurance bespeak the flexibility of the polymer film. In order to evaluate the flexibility, the made films were subjected to folding endurance research. The numerous of bend a film can sustain without interruption will dictate its folding endurance. The computed measured determined were above 125 in all of the generated layers and are noted in **Table No. 16** and it was in the range of 126-178 for all the generated films. Regardless of polymer concentration applied, all the films presented nice folding endurance, bring out that the made films were having the capability to produce hold up the mechanical pressure along with nice flexibility. The folding endurance is a significant evaluation, which assure the flexibility of the generated films. Larger the folding endurance values better will be the flexibility of the films. 6% film (NF3) presented good folding endurance, because of that ensuring good flexibility.

Table No. 5: Optimization of nitrocellulose film former

Nitrocellulose Concentration (%w/v)	1	2	3	4
Thickness (μm)	58 ± 0.06	59± 0.02	59 ± 0.04	60 ± 0.03
Folding endurance	154	131	176	176
Tensile strength (Kg/cm^2)	2.58 ± 0.03	2.60 ± 0.04	2.63 ± 0.07	2.57 ± 0.04

B) Water Resistance

This is the determined of the opposition towards water permeability of the layer. This was done by applying uninterrupted layer on a surface and plunge it in water. The weight before and after immersion was noted and maximize in weight was determined. Large maximize in weight low the water opposition. Here Nitrocellulose Film of 6% (NF3) has relatively, low weight and has the better water resistance. The data were shown in **Table No. 17.**

Table No. 6: Water (W) resistance of nail lacquers

Formulation code	W_1(g)	W_2(g)
NF1	6.86	6.92
NF2	6.84	6.93
NF3	6.89	6.90
NF4	6.93	7.15
NF5	6.82	6.92
NF6	6.85	6.92
NF7	6.90	6.95
NF8	6.92	7.05

Having a base on above studies it was distinct that, NF3 formulation has the excellence properties needful for a nail lacquer and thence 6% w/v of nitrocellulose and 10% w/v of Propylene glycol was determined to be the optimum concentrations.

6 Evaluation of nail lacquer

All preparations presented coveted layer make, smoothness of flow was nice. Coveted quantity of nonvolatile substance (31-41%) was observed with complete evaporation of volatile matter leaving a thin layer; Conclusion were plotted in **Table No. 18.** Drying time was obtained within 52-127 sec. Demur for F2, where it presented 127 sec, all formulation showed fast drying rate. That is less than 60 seconds. The numerous amount were shown in **Table No. 19.**

A) Nonvolatile content

The non-volatile content of all formulation has been shown in the **Table No. 18,** given below

Table No. 7: Nonvolatile content of nail lacquers.

Formulation code	Non-volatile content (%)	Formulation code	Non-volatile content (%)
F0	34 ± 0.38	F6	38 ± 0.81
F1	34 ± 0.38	F7	37 ± 0.70
F2	42 ± 0.81	F8	33 ± 0.40
F3	40 ± 0.40	F9	36 ± 0.41
F4	38 ± 0.81	F10	34± 1.22
F5	38 ± 0.71	F11	38 ± 0.81

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B) Drying time

Table No. 8: Drying time of nail lacquers

Formulation code	Drying time (sec)	Formulation code	Drying time (sec)
F0	51	F6	57
F1	53	F7	60
F2	129	F8	57
F3	53	F9	60
F4	59	F10	59
F5	60	F11	58

C) Smoothness of flow and Gloss:

Both these parameters was obtained to be acceptable as can be received. The nail lacquer dipped onto the glass plate was obtained to dispersed and resultant in unvarying smooth layer. The gloss of the applied lacquer was worthy of comparison with marketed cosmetic test sample achieving the cosmetic credence.

D) Viscosity

The viscosity of the test sample ranged from 100 to 220 centipoise it was obtained that between140to160 centipoise the product was clean and glossy. Furthermore this viscosity range furnished nice attachment and flow property. Viscosity outside this range generate translucence and minimize gloss which will not be cosmetically satisfactory.

Table No. 9: Viscosity of nail lacquers

Formulation code	Viscosity	Formulation code	Viscosity
F1	100	F7	200
F2	111	F8	140
F3	122	F9	142
F4	133	F10	146
F5	184	F11	146
F6	198		

E) Adhesive strength

The adhesive strength of the implied batch was shown to be worthy of comparison with marketed sample and thence can be arrived to exhibit equal adhesive strength on applied nail surface.

Table No. 10: Adhesive strength of nail lacquers

Formulation Code	Force of Adhesion (N)	Adhesive strength (N/m^2)
F11	0.6	12.6
MARKET SAMPLES	0.7	16

F) Percentage drug content determination

Percentage drug ingredients for all the lacquers were obtained to be satisfy and in between 86.25-99.01% which is shown in **Table No. 11.** Largest % of drug constituents was obtained to be 99.01% (F11) and the smallest % of drug content was 86.25% (F3). Drug content more than 90% in the Preparation shows the large no. of quantity of drug present in the Preparation, Confirming that the methods of preparation and the constituents choose are not poignant the stability of drug. Large drug constituents also show to confirm that, a nice curative result can be arrived.

Table No. 11: Percentage drug content

Formulation Code	Drug content (%)	Formulation code	Drug content (%)
F0	90.01	F6	89.38
F1	91.52	F7	90.13
F2	93.76	F8	98.02
F3	86.27	F9	98.24
F4	94.30	F10	97.56
F5	95.82	F11	99.03

G) Diffusion studies across artificial membrane

eISSN 1303-5150

Diffusion research of all the preparations were obtained by artificial membrane (cellophane membrane -0.8μm) for 48 hrs. The diffusion studies were made on all formulations as per shown in above table.

The top formulated batch F0 did not dwell of any permeation enhancers and in vitro diffusion revealed that only 27.10% drug released till 48 hrs. Thus trials were planned to incorporate a permeation enhancer. Salicylic acid at concentrations of 5% (F1), 10% (F2), 15% (F3) and 20% (F4) was tested out. The diffusion studies shown that only 64.18%, 65.10%, 68.34% and 69.10% respectively was obtained in 18 hours. It was clean that salicylic acid has valuable the drug permeation due to its keratoytic activity. But it was also determined that the drug permeation was not yet done and some maximize in salicylic acid concentration is not arrived to valuable permeation. Thence it was declared to choose 15% w/v of salicylic acid as the optimum concentration.

To further improve drug diffusion it was decided to include 2-H-β-CD in concentration of 5% (F5), 7.5% (F6) and 10% (F7) into formulations. The drug release and diffusion across membrane was found to improve in presence of 2-HP-β-CD. At concentration of 5%, 82.40% diffusion in 28% hour was observed. In case of F6, 89.0% diffusion as observed at 28th hours. It was also observed that as concentration of 2-HP-β-CD increased drug diffusion also improved drastically as clear from almost complete drug diffusion of 98.40% release in 20th hour with 7.5% concentration.

Though, inclusion of 2-H-β-CD has improved drug diffusion to 98.40%, it was observed that the release was found to be complete within 20 hours. Therefore to sustain the drug release over an extended period it was decided to include a rate controlling polymer ethyl cellulose at concentration of 0.25% (F8), 0.5% (F9) and 0.75% (F10) and 1.0% (F11) into formulation. The result showed an extended and completed release of 96.80% at 28th hr. in F8 and 93.0% till 36th hour in F9. In F10, a drug diffusion of 97.20% was observed at 40th hr. And finally when the concentration of ethyl cellulose was increased to 1% in F11, a drug diffusion of 98.12 percent which sustained over a period of 48 hours was achieved.

The formulation F11 was selected as the optimized nail lacquer formulation based on drug diffusion studies.

Table No. 12: Comparative study and optimization of salicylic acid concentration

Time (hr)	PERCENTAGE DRUG RELEASE (μg/ml)			
	F1	F2	F3	F4
0	0	0	0	0
2	9.82	11.24	13.38	15.28
4	10.20	12.08	14.99	16.89
6	13.28	14.37	16.37	17.26
8	16.44	17.89	18.87	20.15
10	26.60	28.97	32.06	30.38
12	32.47	36.35	40.23	36.17
16	43.15	42.32	48.39	42.97
20	48.25	49.99	51.83	50.12
24	49.67	50.83	52.62	54.35
28	52.57	54.91	56.85	58.40
32	56.28	58.77	59.35	60.23
36	58.98	59.99	61.29	63.47
40	60.18	62.19	63.95	66.25
44	62.55	63.27	65.97	68.86
48	64.20	65.16	68.37	69.13

eISSN 1303-5150

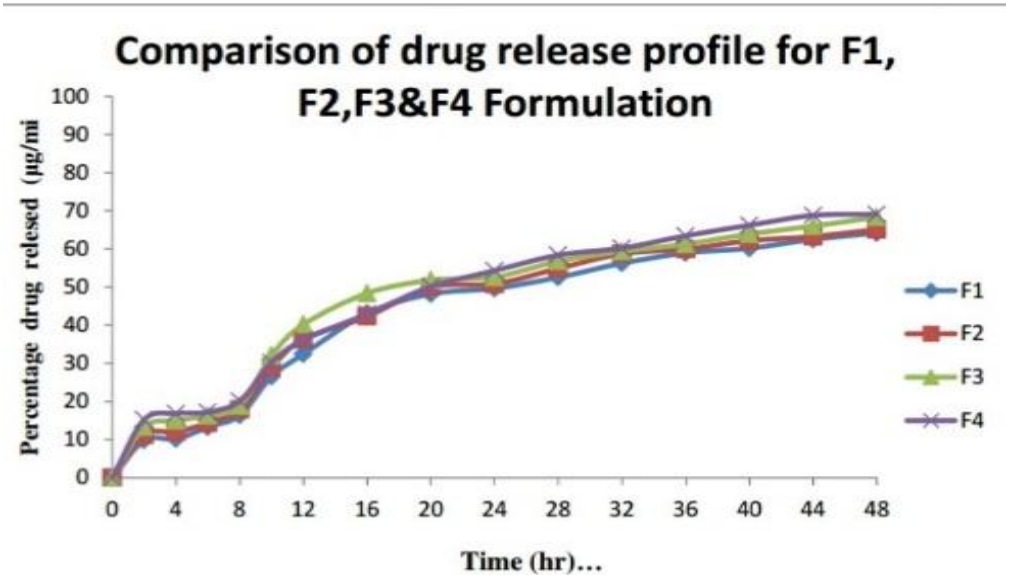

Figure 10: Comparative Dissolution profile of F1 v/s F2 v/s F3 v/s F4

Table No. 13: Comparative study and optimization of 2-HP-β-CD concentration

Time (hr)	PERCENTAGE DRUGT RELEASE		
	F5	F6	F7
0	0	0	0
2	26.27	32.14	39.34
4	32.26	43.57	49.87
6	38.53	52.84	59.67
8	46.53	61.66	67.73
10	48.23	69.36	76.46
12	56.29	76.26	85.06
16	65.16	80.03	92.16
20	76.46	83.36	98.42
24	79.96	88.97	96.26
28	82.42	89.08	94.26
32	80.26	86.35	93.17
36	79.46	84.17	91.84
40	77.33	82.18	90.08
44	76.66	80.88	89.18
48	74.77	78.27	88.99

eISSN 1303-5150

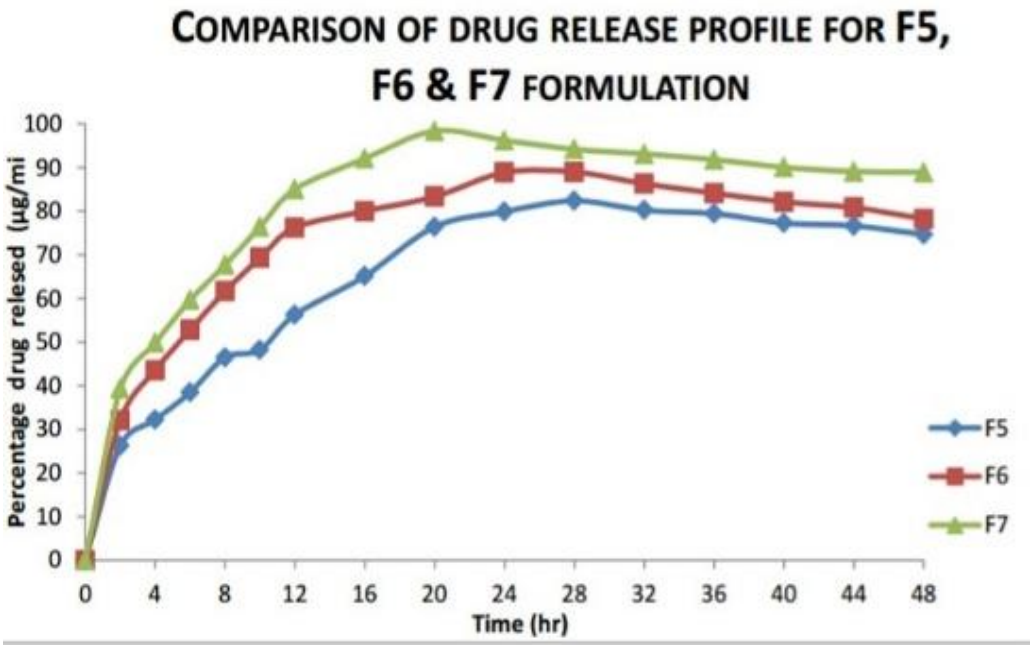

Figure 11: Comparative Dissolution profile of F5 v/s F6 v/s F7

Table No. 14: Comparative study and optimization of Ethyl cellulose concentration

Time (hr)	PERCENTAGE DRUG RELEASE (µg/ml)			
	F8	F9	F10	F11
0	0	0	0	0
2	29.67	26.57	19.45	12.86
4	34.14	31.97	30.47	27.13
6	45.57	40.44	36.92	28.32
8	51.17	44.92	48.85	32.73
10	62.36	53.23	50.75	46.26
12	69.76	60.14	56.80	50.22
16	75.94	68.67	60.25	58.67
20	88.46	72.33	65.72	60.22
24	93.26	83.47	72.68	68.13
28	96.82	89.77	80.52	70.23
32	95.06	95.85	85.73	78.86
36	94.58	93.79	90.63	84.16
40	93.15	90.73	97.57	88.86
44	90.77	89.88	94.23	90.26
48	89.05	88.75	91.33	98.14

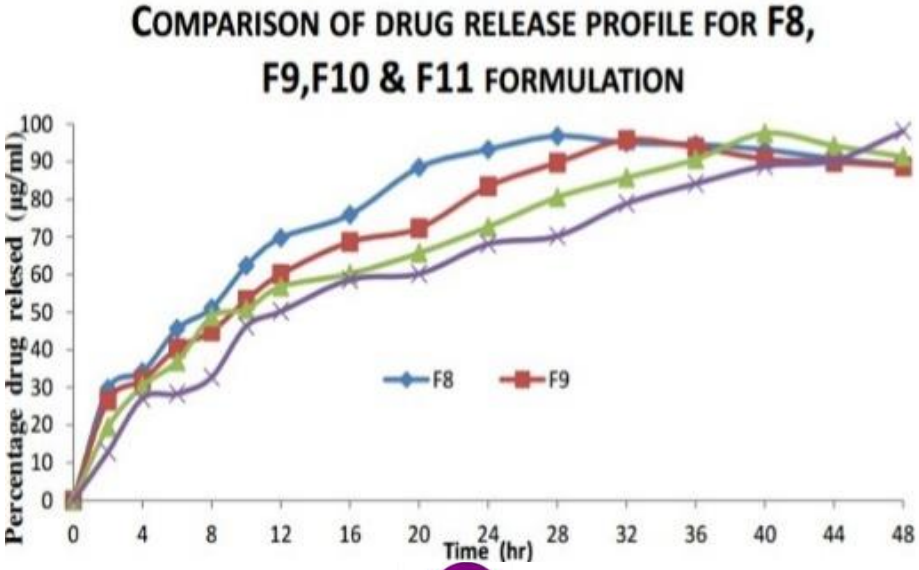

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Figure 12: Comparative Dissolution Profile of F8 v/s F9 v/s F10 v/s F11

H) In vitro ungual permeation studies

To excite and constituting an imitation diffusion research with that of in vivo conditions, i.e. across nail plate, a diffusion study across hooves resultant form freshly slaughtered cattle was done. There was no importance difference and drug release data obtained across artificial hoof's membrane. This research achieve sureness which is nice in vitro in vivo correlation can be demur.

Table No. 15: Comparison of drug diffusion across artificial membrane and hoof's membrane

Time	PERCENTAGE DRUG RELEASE (μg/ml)	
	Drug diffused through artificial membrane	% drug diffused through hoof's membrane
0	0	0
2	12.83	14.51
4	27.13	20.91
6	28.32	26.46
8	32.73	36.76
10	46.26	47.91
12	50.22	56.73
16	58.66	60.44
20	60.21	65.83
24	68.12	72.56
28	70.23	80.61
32	78.86	85.06
36	84.16	89.26
40	88.86	92.32
44	90.26	95.05
48	98.13	97.46

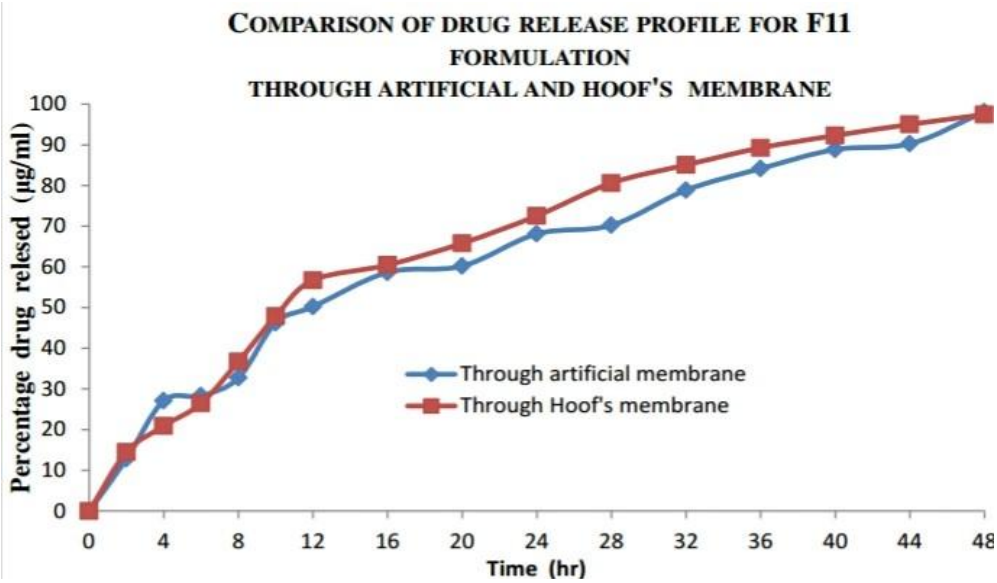

Figure 13: Comparison of drug diffusion across artificial membrane and hoof's membrane

I) ANTI-MICROBIAL STUDY

The zone of inhibition for the many preparation was investigated, and it was obtained range from 17-22mm, which is allow to compare with that standard with 21mm. The show that all the formulations were sensitive to the microorganisms Candida albicans. Conclusion are shown in

Table No. 16: Zone of inhibition of Miconazole nitrate Nail lacquers

Formulation Code	Zone of Inhibition (mm)	Formulation code	Zone of Inhibition (mm)
F1	23	F7	19
F2	19	F8	25
F3	22	F9	18
F4	23	F10	24
F5	18	F11	23

eISSN 1303-5150

F6	17	Standard	22

J) Stability studies

Stability studies were applied to obtain the shelf life and storage condition of a product. In this determination F11 were subjected to speed up stability studies for as per day of 1 month. Stability studies were performed in according to ICH guidelines with importance adjustments.

The studies were obtained to ascertain the changes in physical properties such as Non-volatile content, Drying time, % drug content, drug diffusion at three different conditions f higher temperature (40 ± 2^0C) for 1 month. The conclusion are shown in

Table No. 17: Stability studies data of F11

Parameter	Initial	After
Non content	36±0.82	35±0.36
Dryintin (sec)	57	59
Drug content	99.04	98.52

Table No. 18: Invitro Diffusion profile of F11 upon stability studies

Time	PERCENTAGE DRUG RELEASE (µg/ml)	
	Before stability	After stability
0	0	0
2	12.83	10.61
4	27.13	24.91
6	28.32	26.44
8	32.73	30.26
10	46.26	39.96
12	50.22	45.76
16	58.66	52.56
20	60.21	58.82
24	68.12	62.51
28	70.23	72.06
32	78.86	76.82
36	84.16	81.27
40	88.86	90.54
44	90.26	92.21
48	98.12	97.75

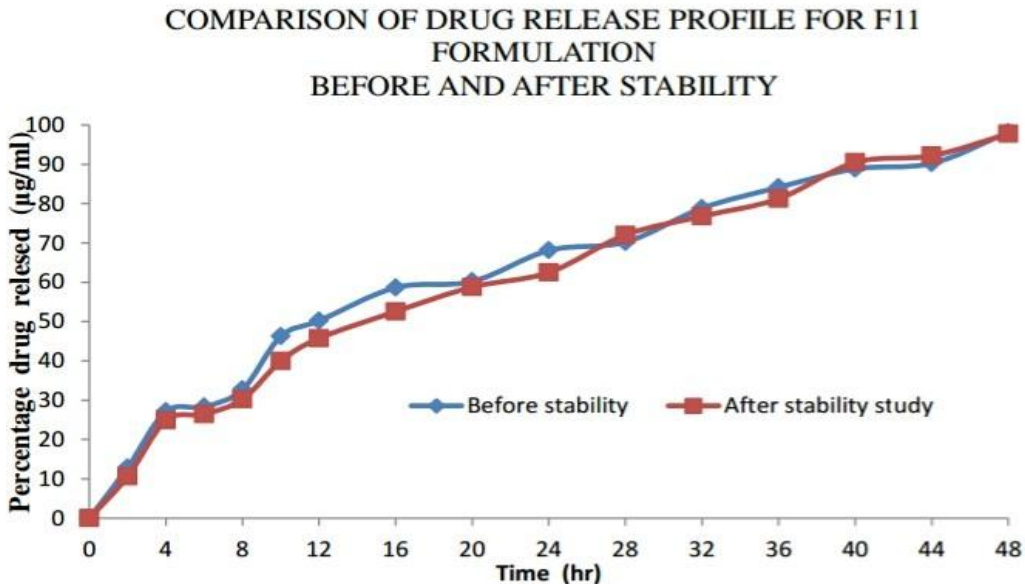

Figure 14: In -vitro diffusion profile of F11 upon stability studies

Theevaluation of formulation after stability study presented there was no important change with respect Non-volatile content, Drying time % drug content and drug diffusion with respect to result obtained before stability charging. Thence it was received that the formulation were obtained to acceptable stability compliance needful as per ICH guidelines.

CONCLUSION

The primary goal of the current research was to develop and assess a miconazole nitrate nail lacquer as an oral medication delivery system for the treatment of onychomycosis. Salicylic acid was used in the production of the medicine chosen, miconazole nitrate. And as a consequence of the FTIR research, the preparation included the medicine and its additions. The microbiological analysis demonstrated that the formulations are responsive to the necessary volatile components. The preparations are susceptible to the Candida albicans fungus. confirmed by the study of microbes. The preparation was stable after being kept at 400°C for a month. In vitro in vivo correlation can be accepted, according to a study on in vitro permeation. The in vitro tests that showed formulation F11 gave a full drug release that sustained over 48 hours lead to the conclusion. Salicylic acid was included in the F11 formulation at a concentration of 15% w/v as a keratolytic agent and at a concentration of 10% w/v as a permeation enhancer. A complete and prolonged drug release was achieved by combining a permeability enhancer and a keratolytic agent, as evidenced by the results of the experiment. Based on optimization and drug diffusion experiments, the formulation of F11 was selected as the nail lacquer formulation. After the stability test, which the stability research validated, there was no longer any interchangeability in the values. The preparations were found to have reached the essential stability conformity with ICH criteria. According to the aforementioned research, medicated lacquers have been demonstrated to be a good base for the ungual drug delivery of an antifungal in the treatment of onychomycosis, which is applied in the treatment of nail infections. Medicated nail lacquers can also be used for glowing and glamorous of nails with easily and time-consuming useful for application, which increases patient compliance.

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eISSN 1303-5150