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Formulation and Evaluation of an Antifungal Nail Lacquer for Onychomycosis

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ABSTRACT

In the present work, a medicated antifungal nail lacquer had been developed. The formulation objective was to provide a sustained release of antifungal over extended period of time, so as to reduce frequency of administration, improve clinical efficacy and improve the patient compliance. The nail lacquer formulation was prepared by simple mixing and analyzed for non volatile content, gloss, smoothness to flow, drug diffusion studies, drug content estimation, anti microbial studies. Among all formulation, nail lacquer prepared with 2% drug, 3% nitrocellulose, 0.5% ethyl cellulose, 20% salicylic acid, 5% propylene glycol and 5% urea in H_2O_2 exhibited good non volatile content, gloss, smoothness to flow, drug release, drug content estimation and antifungal activity. The drug release could be extended up to 48 hr and a complete release of 96.03% was observed. FTIR studies revealed that drug and excipients are compatible. Stability study of selected optimized formulation was done as per ICH guidelines for 1 month, which revealed that no significant change with respect to the evaluations conducted before stability charging. The inhibitory zones for the nail lacquer were evaluated by testing against the *Candida albicans* on Sabouraud's Dextrose Agar. The effective zones of inhibitions suggested miconazole nail lacquer exhibiting moderate antifungal activity for the onchomycosis treatment.

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Introduction

Over the years, the importance of nail permeability to topical therapeutics has been realized, primarily in relation to the treatment of onchomycosis; a fungal infection of fingernails and toenails which affects approximately 19% of the world population and is responsible for approximately 50% of all nail disorders[1]. Topical therapy is highly desirable because of its non-invasiveness, ability to target drugs to the site of action, minimizing systemic adverse effects and improving patient compliance. Recent advances in ungual delivery technology have led to the introduction of antifungal nail lacquers. Miconazole is a broad spectrum antifungal agent of the is a broad spectrum antifungal agent class. Its chemical name is (RS)-1-(2-(2, 4-Dichlorobenzyloxy)-2-(2, 4-dichloro phenyl) ethyl) -1H[2]. It is used in the treatment of superficial and systemic fungal infections caused by *Aspergillus*, *Trichophyton. Interdigitale, Epidermophyton floccossum, Trichophyton violaceum, Mycosporum gypseum, Trichophyton tonsurans* and *Trichophyton soudanense* & *Candida* species[3].

The human nail plate is a much more complex structure than it looks at the first sight. It protects the nail bed, the part directly under the nail plate filled with blood vessels; and the nail matrix, the part at the proximal ventral surface of the nail responsible for cell's proliferation and nail growth. Although thin, the nail plate has 80-90 layers of dead cells and mainly consists of keratins; 4/5 is hard hair-type keratin and 1/5 is soft skin-type keratin[1]; and is mainly associated with stem cell function[4].

The present work investigated the permeability of the antifungal drug, Miconazole through the human nail plate from the nail lacquer formulation with different penetration enhancers. Medicated nail lacquer after application leaves an occlusive film over the nail, which act as a drug depot from which sustained release of antifungal is provided for entire duration of therapy. Finally the formulations were discussed in respect to their enhancement factors by estimating the zone of inhibition against the dermatophyte, *Candida albican*[5].

Materials and Methods

Miconazole nitrate was obtained as a gift sample from Medical Products of India, Mumbai, India; which was chosen as the model antifungal agent. Salicylic acid, thioglycolic acid, urea and hydrogen peroxide was purchased from nice chemicals Pvt. Ltd Cochin. Ethyl cellulose from Kemphasol, Popatwadi, Mumbai. Other chemical used were analytical grade.

Nail lacquer is prepared in 10 different formulations. In all ten formulations the weight of Miconazole (2%) is kept constant. In formulation F1, F3, F4, F5 different concentrations of salicylic acid is used. F6 and F7 enhancer used is 5% and 10% urea in H_2O_2. 5% propelene glycol was added to formulation F8. To sustain the action of drug release. Next study were tried to sustain drug release and provide drug action for extended period. For the same, ethyl cellulose at concentration of 0.25% (F9) was formulated. To further sustain the drug release, the ethyl cellulose concentration was increased to 0.5% (F10), which sustained the drug release to 48th hrs.

The mixture of Miconazole nitrate and nitrocellulose was dissolved in ethyl alcohol in the required quantity using a magnetic stirrer at a constant speed. To above clear solution required quantity of salicylic acid, thioglycolic acid, urea in H_2O_2 and propylene glycol were mixed thoroughly and made up to the volume to 100ml. The prepared nail lacquer was transferred to a narrow mouthed, plastic screw capped glass bottle[6].

Evaluations of Nail Lacquers

Nonvolatile content

10 ml of sample was taken in a petri dish and initial weights were recorded. The dish was placed in the oven at 105^{0}C for 1hr, the petri dish was removed, cooled and weighed. The difference in weights was recorded. Average of triplicate readings was noted[7-9].

Drying time

A film of sample was applied on a petri dish with the help of a brush. The time to form a dry-to- touch film was noted with the help of stop watch[7-9].

Smoothness to flow

The sample was poured from a height of 1.5 inches into a glass plate and spread on a glass plate and made to rise vertically[7-9].

Gloss

Sample of nail lacquer was applied over the nail and gloss was visually seen, compared with marketed cosmetic nail lacquer[7-9].

Drug content estimation

Nail lacquer equivalent to 200mg was dissolved in 50 ml phosphate buffer solution of pH 7.4. Then the solution was ultrasonicated for 15 mints. The resulting solution was filtered, made up to 100 ml with phosphate buffer solution of pH 7.4. From the above solution take 10ml and made up to 100ml with PBS of pH 7.4. Then the diluted solution was estimated spectrophoto-metrically at wavelength of 223 nm and determined the drug content.

Diffusion studies across artificial membrane

Diffusion studies were performed by Franz diffusion cell using artificial membrane (cellophane) of 0.8µm. The membrane was soaked for 24hrs in solvent system and the receptor compartment was filled with solvent. Nail lacquer equivalent to 200mg was applied evenly on the surface of the membrane.

The prepared membrane was mounted on the cell carefully to avoid entrapment of air bubbles under the membrane. The whole assembly was maintained at 37°C, and the speed of stirring was kept constant for 20hrs. The 5ml aliquot of drug sample was taken at time intervals of 2hr, 4hr, 6hr, 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 estimation. Each experiment was repeated thrice[7-10].

In vitro ungual permeation studies

Hooves from freshly slaughtered cattle, free of adhering connective and cartilaginous tissue, were soaked in distilled water for 24hrs. Membranes of about 1mm thickness were cut from the distal part of hooves. *In vitro* permeation studies were carried out by using Franz diffusion cell, the hoof membrane was placed carefully on the cell. Then the nail lacquer equivalent to 200mg was applied evenly on the surface of the nail membrane. The receptor compartment was filled with solvent phosphate buffer solution of pH 7.4, and the whole assembly was maintained at 37°C with constant stirring for 48hrs. The 5ml aliquot 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 and was replaced by the fresh solvent. The drug analysis was done by using double-beam UV spectrophotometer at 223nm[8-11].

Determination of antimicrobial activity

Candida albicans were employed for testing antifungal activity using the cup-plate method. The culture was maintained on sabouraud's agar slants. 20 ml of melted sabouraud's agar medium was inoculated with 72 hrs old 0.2 ml suspension of *Candida*

albicans in the Petri dish and allowed to set by keeping undisturbed for 15 mints. The cups (10mm diameter) were punched in the Petri dish and filled with 0.05 ml of a solution of the sample dissolved in DMSO. The plates were kept for diffusion at +40^{0}C for 1hr, and incubated at 30^0 C for 48 hrs. After the completion of incubation period the zone of inhibition in millimeter were measured. Along with the test solution in each petri dish one cup was filled up with solvent, which act as control. The zone of inhibition was recorded and compared with control[12,13].

Stability study

Stability studies of nail lacquers were carried out as per ICH guidelines. Samples were stored at temperature of 25±2 ^{0}C/60 ± 5% RH for 6months and 40 ± 2°C/75 ± 5% RH for 1 month. Then the samples were analyzed for non volatile content, drying time, gloss, and smoothness of flow, drug content and diffusion across artificial membrane[811].

Results and Discussion

The objective of the present study was to formulate a nail lacquer for inhibiting fungal growth on or underneath toe nails or finger nails so that the appearance of the nails is improved. Formulation includes a film former nitrocellulose, permeation enhancers such as Urea in hydrogen peroxide and propylene glycol, salicylic acid as keratolytic agent and an antifungal agent (Miconazole nitrate) in ethanol. Formulation is prepared by simple mixing method.

All formulations showed desired film formation, good smoothness of flow, drying time was found to be satisfactory, except the formulation F2 and gloss was satisfactory. Desired amount of nonvolatile matter was seen with complete evaporation of volatile matter leaving a thin film, it range from 32-41%.

Drying time of the lacquers was found in range of 54-68sec, except F2. Formulation F2 showed an increase drying time because the used permeation enhancer, thioglycolic acid decreases the volatility of the prepared lacquer.

Smoothness of flow and Gloss were found to be satisfactory except formulation F2 because of the sticky film forming nature of thioglycolic acid when compared with marketed cosmetic lacquers.

Percentage drug content for all the lacquers was in between 90-99% .Drug content more than 90% in the formulation shows the high amount of drug present in the formulation, without causing any change in the ideal property of ideal lacquers.

The *in vitro* diffusion studies showed very good release of 96.19% in the 20th hrs in F8. This was due to the hydration and permeation property of propylene glycol which improved permeation of the drug. The film characteristics, smoothness to flow and drying time were also better for this formulation.

Now the study were tried to sustain drug release and provide drug action for extended period. For the same, ethyl cellulose at concentration of 0.25% (F9) was formulated. The result showed an extended and complete drug release of 94.25% at 36th hrs. To further sustain the drug release, the ethyl cellulose concentration was increased to 0.5% (F10), which sustained the drug release to 48th hrs. A complete drug release of 96.03% was observed at 48th hrs. The same was selected as the optimized nail lacquer formulation.

In vitro ungual permeation studies were carried out using bovine hoof membrane. Among the various formulations prepared, F10 exhibited optimum diffusion across artificial membrane and hence the same was selected for *in vitro* ungual permeation studies across bovine hoof membrane. The results showed a

superimposable diffusion release profile. This further proved that artificial cellophane membrane mimicked the characteristic features of ex vivo bovine hoof membrane.

The zone of inhibition for the various formulations was determined, and it is found to range from 17-22mm, indicating that the formulations were sensitive to the organisam.

The evaluation of formulations after stability charging showed there was no significant change in physical appearance and colour. Non volatile content, drying time, smoothness of flow, gloss along with changes in % drug content and drug release study showed results obtained before stability charging. Thus the results of stability study showed that there are no stability problems. The formulations were found to be stable for a period of 1 month at 40^0C.

Conclusion

The Miconazole nail lacquer was found to be effective in inhibiting the growth of the nail fungi, *Candida albicans* and gave desired zones of inhibition conferring for the reapplication of the formulation at intervals for sustaining the certain state of inhibition to cure the fungal infection. The evaluated enhancer, propylene glycol; keratolytic agent urea in H_2O_2 facilitated the Miconazole's permeation and penetration in to the nail plate. The stability tests showed that the formulations were stable at 40^0 C for 1 month.

From all the datas, it can be concluded that medicated nail lacquers can be used as a tool for the ungual drug delivery system in the treatment of onychomycosis. Apart from treating the nail infections, the medicated nail lacquers can be also used for beautification of nails with ease of application. This improves patient compliance and acceptability.

References

1. Vejnovic I, Simmler, L, Betz G. Investigation of different formulations for drug delivery through the nail plate. *International Journal of Pharmaceutics*. 2010; 386:185–194.
2. Gunt HB and Kasting GB. Effect of hydration on the permeation of ketoconazole through human nail plate *in vitro*. *European Journal of pharmaceutical sciences*. 2007; 32:254–260.
3. Rubio MC, Ariz IR, Gil J, Benito J, Rezusta A. Potential fungicidal effect of Voriconazole against *Candida* spp. International Journal of Antimicrobial Agents, 2005;25:264–267.
4. Berker DA, Andre J, Baran R. Nail biology and nail science. *International Journal of Cosmetic Science*. 2007; 29:241-275.
5. Kiran SR, Chandrashekhar B, Vishnu P, Prasad MVV. Ungual drug delivery systems of ketoconazole nail lacquer. *Int J Appl Pharm*. 2010; 4:17-19.
6. Tandel A, Agrawal S, Wankhede S, Transungual permeation of the voriconazole nail lacquer against trichophyton rubrum, *Journal of Drug Delivery & Therapeutics*; 2012, 2(1) :201-215.
7. Patel RP, Naik SA, Patel NA, Suthar AM. Drug delivery across human nail. *Int J Curr Pharm Res* Vol.1, Issue1 2009; 01:01-7.
8. Shireesh KR, Chandra SB, Vishnu P, Prasad MVV. Ungual drug delivery system of ketoconazole nail lacquer. *Int J Appl Pharm* 2010; 02:12-19.
9. P. P. Sharma. Cosmetics- Formulation, Manufacturing & Quality control. 3rd ed. Vandana publications; Delhi; 2005; 467-479.
10. Shirwaikar A. A., Thomas T, Lobo R , Prabhu K. S, Treatment of Onychomycosis: An Update, *Indian J Pharm Sci*. 2008 Nov-Dec; 70(6): 710–714.
11. Philip R. Cohen, MD, Onychomycosis diagnosis, *Indian J Dermatol Venereol Leprol*. 2012; 78:263-270.
12. B. Mahesh and S. Satish, Antimicrobial activity of some important medicinal plant against plant and human pathogens, *World Journal of Agricultural Sciences*; 2008, vol. 4 (S), pp no: 839- 843.

13. Kiran, S., and Shekar C, Ungual drug delivery system of ketoconazole nail lacquer. *In vitro*, 2010; 1-3.Chandra R, Kumar S, Aggarwal A. Evaluation of Nail Lacquer. 2012;2(4):379–82.

Table 1. Composition of nail lacquer

Ingredients	F0	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Miconazole nitrate (% w/w)	2	2	2	2	2	2	2	2	2	2	2
Nitrocellulose (% w/v)	3	3	3	3	3	3	3	3	3	3	3
Ethyl cellulose (%w/v)	….	….	….	….	….	….	….	….	….	0.25	0.5
Salicylic acid (% w/w)	….	10	…	15	20	25	20	20	20	20	20
Thioglycolic acid (% w/v)	….	….	10	….	….	….	….	….	….	….	….
Urea in H_2O_2 (% w/v)	….	….	….	….	….	….	5	10	10	10	10
Propylene Glycol (% w/v)	….	….	….	….	….	….	….	….	5	5	5
Ethanol (ml)	100	100	100	100	100	100	100	100	100	100	100

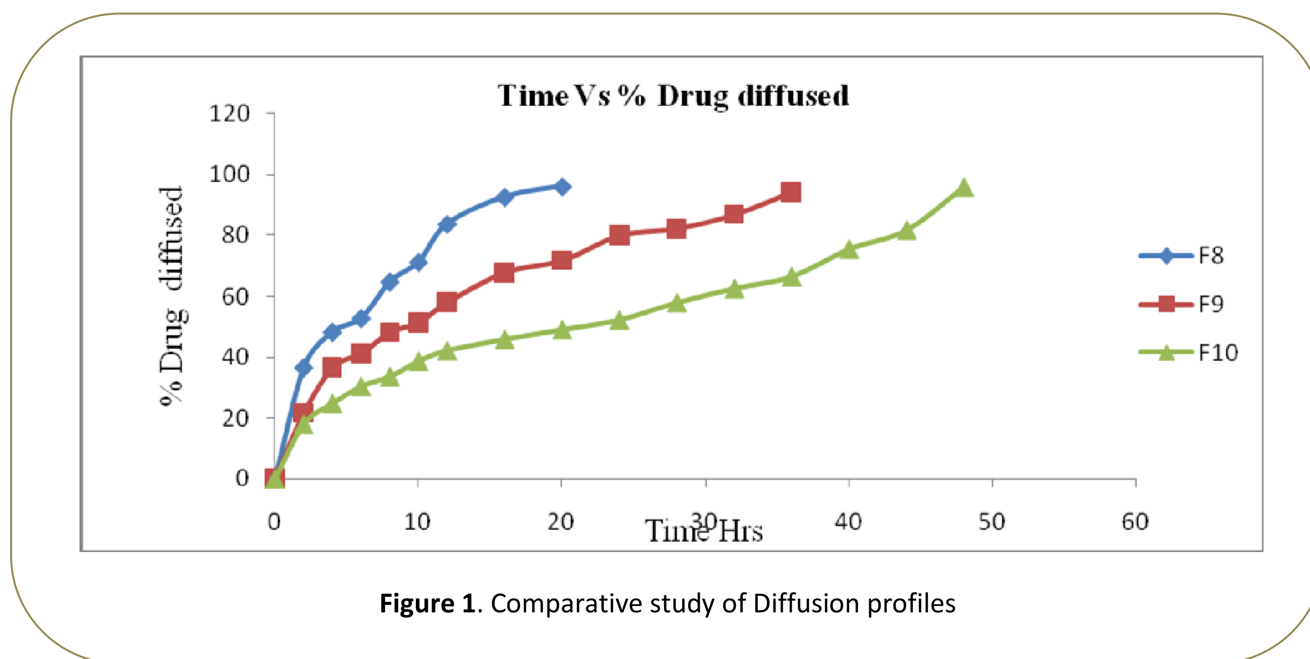

Figure 1. Comparative study of Diffusion profiles

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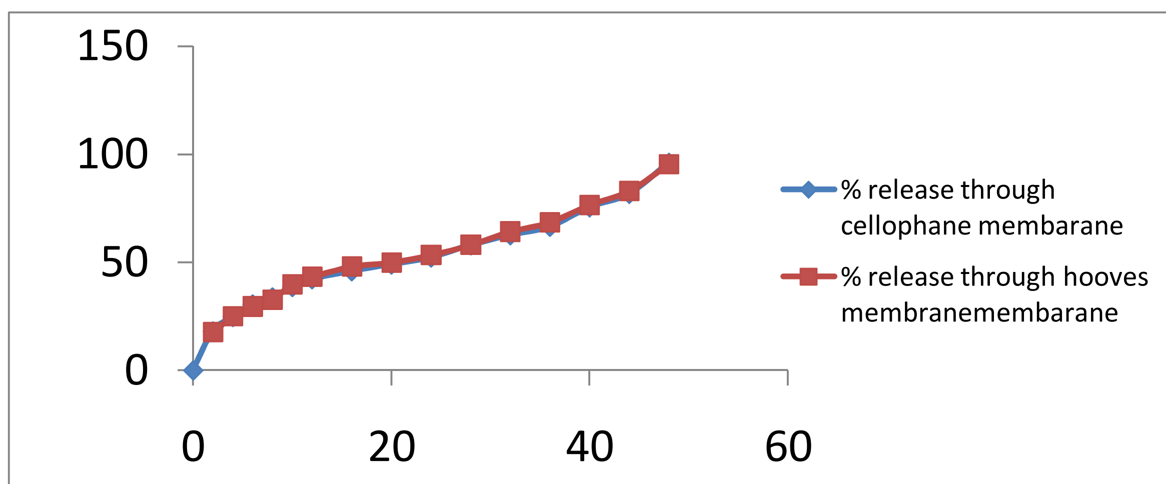

Figure 2. Diffusion of F10 across cellophane and hooves membrane