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Research Paper

A NOVEL TRANSUNGUAL FORMULATION (NAIL PATCH) FOR DELIVERY OF CICLOPIROX OLAMINE INTO THE NAIL AND THE NAIL FOLDS

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The current work describes (1) development of a transungual patch containing ciclopirox olamine (CPO) and the penetration enhancers (thiourea and propylene glycol); and (2) comparative evaluation of patch against commercially available topical formulation (Penlac® nail lacquer) for drug delivery into the nail folds and across the nail plate. An immediate release nail patch containing ciclopirox olamine, thiourea and propylene glycol in a HPMC matrix, with a peripheral lining of acrylate PSA (DURO-TAK® 87-4287) was prepared. The *in vitro* drug release from the transungual patch was evaluated using USP Apparatus 5. Human cadaver skin was used to determine the *in vitro* skin permeation and epidermal accumulation of CPO using the nail patch and Penlac® nail lacquer. The *in vitro* transungual permeation and ungual delivery of CPO from the two formulations were evaluated using human cadaver toenails. The *in vitro* release profile shows greater than 80% of drug release within 2 h indicating that the drug release is not the rate limiting step in drug delivery. The nail patch showed significant increase in epidermal accumulation (2.8 fold), transungual permeation flux (4.8 fold), drug concentrations in the receiver compartment (5.2 fold) and in the nail (2.7 fold) compared to the commercial formulation.

Keywords: Transungual, HPMC, Ciclopirox olamine, Onychomycosis, Penetration enhancers, Antifungal

INTRODUCTION

Topical drug delivery of antifungal agents for treatment of onychomycosis is challenging due to the limited permeability of drugs across the nail plate (Murdan, 2008). Penlac® nail lacquer (ciclopirox) and Loceryl® nail lacquer (amorolfine) are the two commercially available topical formulations for treatment of onyochomycosis. The design to the nail lacquer formulations containing a hydrophilic layer have the advantage of increased contact with the nail and efficient drug delivery (Monti *et al*., 2005). However, there is limited success in passive transungual drug delivery of antifungal agents. One approach to improve the passive drug delivery into and across the nail is use of chemical penetration enhancers.

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Herein, we describe an extension of our previous work of selection of penetration enhancers for delivery of ciclopirox olamine (CPO) into the two target tissues - nail plate and nail folds (Palliyil *et al.*, 2013). Present work aims to develop a transungual formulation containing the selected penetration enhancers and to evaluate its performance.

The key properties (Murdan, 2008) of a successful topical formulation for treatment of onychomycosis are:

- Ease of use.
- Maintain adequate contact with nail.
- Easy release and diffusion of drug from the formulation.
- High thermodynamic activity.
- Ability to hydrate the nail.
- Presence of penetration enhancers.

It was hypothesized that a well-designed nail patch formulation can meet the above criteria for a topical formulation. Nail patches can help to overcome some of the limitations of the nail lacquer formulation. These limitations include the possibility of drug crystallization in the film after the solvent evaporates from the nail lacquer, the filing of the nail prior to lacquer application and the absence of penetration enhancer, etc. (Murdan, 2008). Thus, this work is also a reformulation strategy for CPO to attain a better transungual drug delivery compared to the existing formulation Penlac® nail lacquer.

MATERIALS AND METHODS

Ciclopirox olamine (CPO) was purchased from Haorui Pharma-chem Inc. (Edison, NJ, USA). Propylene glycol (PG) was obtained from MP Biochemicals. Thiourea (TU) and sodium phosphate dibasic were obtained from Acros Organics. Potassium phosphate monobasic, sodium hydroxide, gentamicin sulfate, dimethyl sulfate and triethyl amine were bought from Sigma Aldrich, USA. The polyacrylate Pressure Sensitive Adhesive DURO-TAK® 87-4287 was a gift sample from Henkel, USA. The cellulose ether Hydroxypropyl methylcellulose (HPMC K15 M) was purchased from the Dow Chemical Company (Michigan, USA). The non-occlusive Ethylene Vinyl Acetate (EVA) backing membrane, 3M™ CoTran™ 9707 and the fluoropolymer coated polyester film 3M™ Scotchpak™ 9742 were obtained from 3M (St.Paul, MN, USA). Franz diffusion cells and neoprene nail adapters were purchased from PermeGear. Human cadaver toe nails were purchased from Anatomy Gifts Registry (Hanover, MD, USA). The human cadaver skin was obtained from Allosource (Cincinnati, OH, USA). Stainless steel patch holders were purchased from Agilent. Nanopure water was used for preparation of 10 mM pH 7.4 Phosphate Buffered Saline (PBS). HPLC grade acetonitrile from Fischer scientific was used in preparation of mobile phase. All the chemicals were used as received without any further purification.

High Pressure Liquid Chromatography

CPO content in the samples was assayed using an Agilent1100 series HPLC with dual wavelength detector, using a modified, pre-assay derivatization method (Escarrone *et al.,* 2008; Lehr and Damm, 1985; Myoung and Choi, 2003). The previously reported sample preparation method for CPO was used prior to analysis using HPLC (Palliyil *et al*., 2013). Acetonitrile: water (50:50 v/v) was used as the mobile phase with a 150 mm by 4.6 mm Eclipse Plus C18 column

(Agilent). The flow rate was 1 mL/min and the injection volume was 80 µL. The wavelength of detection for derivatized CPO was 298 nm.

Recovery of Ciclopirox Olamine from Human Nails

The drug recovery from the target tissues was evaluated using the method described in our previous report (Palliyil *et al.,* 2013). Solutions of CPO in pH 7.4 PBS with concentrations 0.0253 mg/mL, 0.0505 mg/mL, 0.202 mg/mL, 0.809 mg/mL, 3.240 mg/mL and 6.470 mg/mL were prepared. Approximately 20 mg of human nail clippings were added to each concentration of CPO solution. Each concentration was studied in triplicate. The nail clippings were treated with 200 µL of each of these solutions for 3 h at 32 ± 1 °C. To dissolve the nails, 200 µL of 1 M NaOH was added to each sample. The nail solutions were diluted to reduce the concentration of 1 M NaOH to 0.1 M and the sample preparation method was followed to quantify the concentration of CPO.

Recovery of Ciclopirox Olamine From Epidermis and Dermis Of Cadaver Skin

A stock solution containing 1.6 mg/mL of CPO in pH 7.4 PBS was prepared. Solutions with the concentrations 0.1 mg/mL, 0.2 mg/mL, 0.4 mg/mL, 0.8 mg/mL, 1.2 mg/mL were prepared using the stock solution. 50 µL of each of these solutions, including the stock contains 5.2 µg, 10.4 µg, 20 µg, 40 µg, 60 µg and 80 µg of CPO, respectively. Each of the above concentrations was studied in triplicate. Skin sections with 9 mm diameter were punched from a piece of human cadaver skin, using a cork screw borer. The epidermis and the dermis were separated for each section, using the modified heat separation method (Palliyil *et al.*, 2013). Both the epidermis and the dermis, for each skin section were separately treated with 50 µL of the drug solutions for 4 h in microcentrifuge tubes. After the drug treatment, 1 mL of the mobile phase was added to each of the microcentrifuge tubes and equilibrated for 24 h at 37 ºC for drug extraction. The extracting medium was filtered through 0.22 µ syringe filters, derivatized and analyzed using HPLC.

Fabrication of Transungual Patch

As shown in Figure 1A, transungual patch in cross-section consists of Backing Membrane (BM), the Pressure Sensitive Adhesive (PSA), drug loaded HPMC film ((CPO+ PEs) $_{HPMC}$) and Release Line (RL). Table 1 gives the composition of the drug loaded HPMC film. The quantities of the components of the matrix were calculated as percentage of dry polymer weight of 0.2 g. CPO, TU and HPMC were weighed into a glass vial. 6 mL of acetone:water (1:1) was added to the vial and vortexed for 2 min. PG was weighed into the glass vial and vortexed for 5 min. The resulting suspension was poured into a disposable petri-dish (60 mm X 15 mm) and air dried overnight. This method of preparation of the hydrophilic matrix is a modification of the literature method (Chandak and Verma, 2008). The process of preparation of the drug loaded HPMC films is shown in step 3 of Figure 1B.

The polyacrylate PSA (DURO-TAK® 87-4287) (15 g) was cast on the release liner (3M™ Scotchpak™ 9742) (15 cm X 15 cm) and air dried overnight. The backing membrane (3M™ CoTran™ 9707) (15 cm X 15 cm) was laid on the dry acrylate film (Step 1, Figure 1B). Discs (60 mm diameter) were cut out from the acrylate films (Step 2, Figure 1B). The release liner was peeled off (Step 6, Figure 1B) and adhesive side of the

Figure 1: A) Design of the Transungual Patch Showing the Layers of the Patch and the Peripheral Adhesive Layer, B) Fabrication of the Prototype Transungual Patch, BM = Backing Membrane, RL = Release Liner, PSA = Polyacrylate Pressure Sensitive Adhesive, $(CPO+PEs)_{HPMC}$ = Hydrophilic HPMC Film Containing CPO, TU and PG

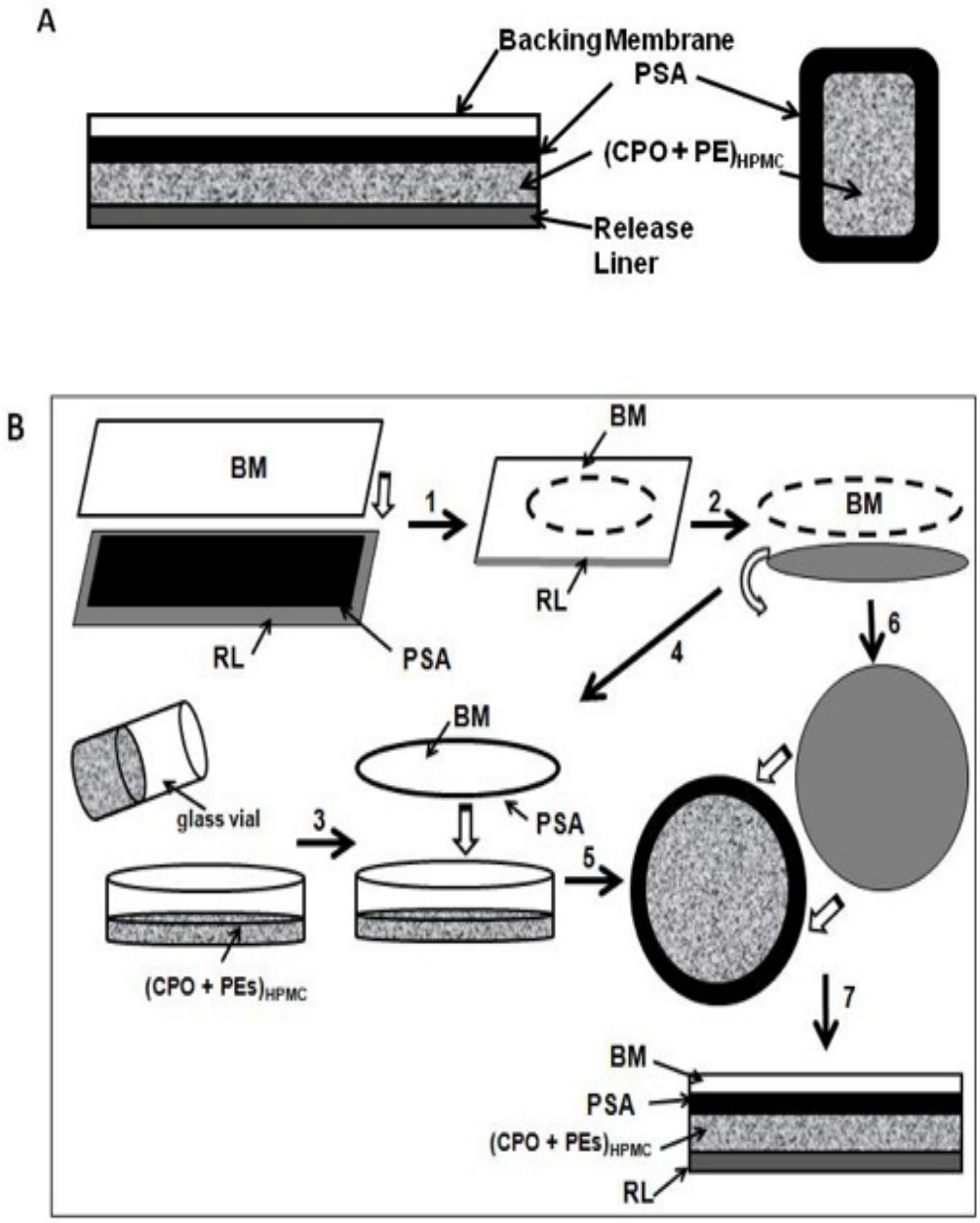


Table 1: Composition of Drug Loaded HPMC Film Containing Penetration Enhancers

HPMC K15M (g)	CPO (% w/w)[a]	TU (% w/w)[a]	PG (% w/w)[a]	Patch Dry Weight (g)	CPO Load (% w/w)[b]
0.2	10	10	150	0.5594	3.83

acrylate film was laid (Step 4, Figure 1B) onto the HPMC film in the petri-dish (Step 3, Figure 1B). This helped to separate the film from the petri-dish (Step 5, Figure 1B). The release liner was replaced on top of the HPMC film to form the complete patch (Step 7, Figure 1B). This method of preparation gave a prototype formulation containing the HPMC layer in the center with a peripheral PSA layer.

In vitro Drug Release Using USP Apparatus 5

The prototype patch was characterized for drug release using the USP Apparatus 5 (Paddle over disc method). Figure 2 shows the components of USP apparatus 5 used in the release study. 500 mL of dissolution medium (pH 7.4 PBS) was added to a standard 900 mL vessel and equilibrated to a temperature of 32 ± 0.5 ºC. The release liner of the transungual patch was removed and the patch was mounted on the stainless steel patch holder (Agilent) that maintains a release area of 10 cm^2. The release surface was placed facing the paddle, in the patch holder (Agilent). The disc was then placed in the bottom of the vessel. The paddle was adjusted to maintain a distance of 2.5 ± 0.2 cm from the drug release surface in the patch holder. The paddle was started immediately at 50 rpm. At predetermined time points of 10, 20, 40, 60, 120 and 240 min, 5 mL of the dissolution medium was withdrawn and replaced with equal volume of fresh buffer. Acetonitrile (0.5 mL) was added to the samples (0.5 mL), vortexed for 5 min, and centrifuged at 10,000 rpm for 10 min to remove HPMC in the sample. CPO in the supernatant was derivatized as described previously. The derivatized samples were filtered through Restek® 0.22 µ syringe filters and analyzed using HPLC. The release kinetics models of zero order, first order and square root of time were fitted to the release data using the model equations 1, 2 and 3 (Gafourian *et al.*, 2007).

$$Q_t = kt \quad ...(1)$$

$$Ln\ (100\text{-}Q_t) = lnQ_0 - kt \quad ...(2)$$

$$Q_t = kt^{1/2} \quad ...(3)$$

In Equation (1) to (3), Q_t is the percent of CPO released at time t and k is coefficient in the equations. In Equation (2), Q_0 is the initial percentage of CPO, which is equal to 100. The goodness of fit was determined using the graphical residuals analysis and the adjusted R^2 values (Microsoft Excel 2007). A normal probability plot (Microsoft Excel 2007) was used to determine the randomness of the residuals and the goodness of the model fit.

In Vitro Skin Permeation

The *in vitro* skin permeation studies were performed in triplicate using Franz diffusion cells with internal volume of 3 mL and orifice diameter of 9 mm. The skin pieces were placed between the donor and the receiver compartment of the Franz cell. The receiver compartment was filled with 3 mL of pH 7.4 PBS and the skin was allowed to equilibrate for 60 min prior to application of the patch. The patches with 9 mm diameter were placed with the release surface in contact with the skin. Penlac® nail lacquer was used as the positive control. Samples (0.5 mL) were withdrawn at 1, 2, 4, 8, 12 and 24 h and replaced with equal volume of fresh buffer to maintain the sink conditions. The cumulative amount of CPO (µg/cm^2) permeating across the skin was calculated and plotted against time to determine the steady-state flux (µg/cm^2/h) and lag-time (h). The steady state flux was calculated from the slope of the linear portion of the plot.

The *in vitro* Skin Accumulation

The CPO levels in the epidermis and dermis were determined by using the heat separation and extraction methods described in our previous report (Palliyil *et al*., 2013). Upon conclusion of skin permeation experiment, the cadaver skin was dismounted from the Franz cell and washed with nanopure water thrice on both the epidermal and the dermal sides to remove any surface drug. The skin was then blotted with tissue paper and wrapped in aluminum foil. Each of the samples was heated in the oven at 45 ºC for 10 min. The epidermis was then peeled from the dermis using sharp forceps. The thickness of the epidermis and the dermis were measured using electronic digital micrometer (Marathon Management). The two layers were then placed in separate glass vials containing 1 mL of the mobile phase each at 37 ºC for 24 h to extract CPO. The concentration of the drug in the extracting medium was quantified using HPLC. The drug levels were reported in µg/mL to determine if the Minimum Inhibitory Concentration (MIC) was achieved in the epidermis and the dermis. The positive control in this study was Penlac® nail lacquer. The enhancement factors, EF_{epi} and EF_{der} give the increase in accumulation of CPO in the epidermis and dermis relative to the control. The enhancement factor for CPO accumulation in the epidermis and dermis were calculated using Equation (4).

$$Enhancement\ Factor,\ EF_{epi/der} = \frac{[CPO]epi/der\ for\ the\ patch}{[CPO]epi/der\ for\ Penalc} \quad ...(4)$$

where $[CPO]_{epi/der}$ is the concentration of CPO in the epidermis or the dermis.

In Vitro Transungual Permeation

The cadaver toenails (Anatomy Gifts Registry) were thawed to room temperature. The nails were washed with nanopure water three times and dried using tissue paper. The toenails were cut into 7 mm X 7 mm pieces in order to minimize the lateral diffusion. Description of lateral diffusion and its impact on transungual drug permeation is beyond the scope of this article. The thickness of the nail pieces were measured at the center using digital calipers. The nail pieces were then hydrated at 100% RH overnight prior to start of experiment. The hydrated nail pieces were placed in the neoprene nail adapters with orifice diameter of 5 mm. The adapters were mounted between the donor and receiver compartment so that the ventral nail surface is in contact with receiving medium. The receiver compartment was filled with pH 7.4 PBS (3 mL) containing 0.1% w/v gentamicin sulfate and the assembly was maintained at 32 ± 1 ºC throughout the duration of the experiment. The nails were equilibrated with the receiving medium for 60 min prior to the application of the first patch. Each sample was tested in triplicate. Penlac® nail lacquer was used as the positive control. The patches with diameter of 5 mm were cut. The release liner was removed and the drug matrix was placed in contact with the nail. The study continued for 30 days. Throughout the duration of the study, the Franz cells were dosed with a new patch daily. The control cells were dosed with Penlac® nail lacquer daily for seven days. On the 7th day, alcohol was used to remove all the layers of the nail lacquer and a new layer applied for the next seven days. This process of lacquer application was based on the instructions in the package insert of Penlac® nail lacquer. On the days 5, 10, 15, 20,

25 and 30, 0.5 mL of the sample was withdrawn from the receiver compartment and replaced with equal volume of new receiving medium. The samples were analyzed for CPO using the HPLC method. Additionally on day 30, the patch and the lacquer were removed from the nails. The nails were dismounted from the nail adapters and the drug content was quantified by separating the nail just below the adapter orifice and the nail surrounding the orifice (Palliyil *et al*., 2013). The drug quantification was performed using the process described in the sample preparation. A plot of cumulative amount of CPO (µg/cm^2) permeating the human nail against time (days) was plotted to determine the lag-time (days) and permeation flux (µg/cm^2/day). The concentration of CPO within the nail was reported as µg CPO/ mg nail.

Statistical Analysis of Data

The experiments were performed in triplicate and the data is reported as the mean (± standard deviation). One way analysis of variance (ANOVA) was used to determine difference in means (Microsoft Excel 2007). A p-value <0.05 was considered as significantly different.

RESULTS AND DISCUSSION

High Pressure Liquid Chromatography

A linear response was attained in the range of 0.09 to 46.73 µg/mL with a correlation coefficient of 0.9996. The retention time, the Limit of Quantification (LOQ) and Limit of Detection (LOD) for the derivatized CPO were 4.6 min, 90 ng/mL and 25 ng/mL respectively.

Recovery of CPO from Human Nail

A recovery of 86-97% was obtained for CPO in the concentration range of 0.25 µg/mg nail to 64.7 µg/mg nail after dissolution in 1 M NaOH and the pre-column derivatization process.

Recovery of CPO from Epidermis and Dermis

74.5 to 95.6% of ciclopirox olamine was recovered when epidermis with a diameter of 9 mm was treated with 5.2 µg to 80 µg of CPO after the extraction of the epidermis. The recovery for the dermis was greater than 95%.

Fabrication of Transungual Patch

The transungual patch is designed such that, the peripheral acrylate PSA will adhere to the skin (2-5 mm around the nail folds) and hold the patch in place throughout the duration of application (24 h). The nail folds and the nail plate will remain in contact with the hydrophilic matrix for a period of 24 h. The patch was designed for once daily application. Table 2 shows the thickness different layers of the transungual patch. The unique design of the patch offers the advantages: (1) Higher concentration of solubilized form of CPO, (2) Presence of PEs selected for the two target tissues – the nail and the nail folds, (3) Increased hydration of the nail plate due to the hydrophilic nature of HPMC; and (4) Separate adhesive layer to improve the adhesiveness to the skin. Hydrophilic polymers have been reported in formulation of transungual delivery systems. A hydrophilic chitosan derivative has been utilized in the development of a water soluble nail lacquer formulation for ciclopirox and amorolfine (Monti et al., 2005; Monti *et al.*, 2010). The hydrophilic layer of the bilayered nail lacquer system containing terbinafine hydrochloride consists of HPMC E15 and penetration enhancer PEG 400 (Shivakumar *et al.*, 2010). A hot melt extrusion approach has been reported for formation of

Table 2: Thickness of Different Layers of Prototype Transungual Patch				
Backing Membrane Thickness (mm ± SD)	Release Liner Thickness (mm ± SD)	PSA Layer Thickness (mm ± SD)	HPMC Film Thickness (mm ± SD)	Total Patch Thickness (mm ± SD)
0.058 ± 0.001	0.087 ± 0.001	0.483 ± 0.055	0.165 ± 0.047	0.649 ± 0.047

hydroxypropyl cellulose (HPC) films containing ketoconazole (Mididoddi and Repka, 2007). However, there are no reports in the literature of a formulation design similar to the prototype patch containing penetration enhancers for transungual and epidermal delivery as discussed in the current project.

In Vitro Drug Release of Transungual Patch

Four individual patches (60 mm diameter) were studied for CPO release using USP Apparatus 5. At 2 h and 4 h the percent CPO released are 84.84 ± 1.74% and 91.99 ± 2.89%, respectively (Figure 3A). Figure 3A shows that the patch formulation design does not hinder the release of CPO and the in-house acceptance criterion of >80 % drug release is met in 2 h for all the four patches tested. This ensures that the drug release is not the rate limiting step in delivery of CPO to the target tissues. Thus the novel transungual patch is an immediate release formulation, designed for application once a day. Figure 3A shows an initial burst release of CPO which is evident at 10 min with about 44% of drug release. The burst release is due to the formation of channels within the matrix, when the hydrophilic polymer (HPMC) was exposed to the dissolution medium and thereby releasing the hydrophilic drug (Chandak and Verma, 2008). A similar burst release of Methotrexate from the transdermal patches consisting HPMC K4M, K15M and K100M has been reported (Chandak and Verma, 2008). The release kinetics models of zero order, first order and square root of time were fitted to the release data using the model equations 1, 2 and 3 (Gafourian *et al.,* 2007). In all the three models, the residuals were random and the probability plot showed that the residuals were close to a straight line. Hence the adjusted R^2 value was used to select the best fit model. It is clear from the values in Table 3 that the release of CPO from the prototype patch follows a first order process.

In Vitro Skin Permeation

The skin permeation for the drug was evaluated for the patch formulation using Penlac® nail lacquer as the comparator product. The steady state flux for the transungual patch was calculated from the slope of the linear portion of the plot in Figure 3B. Similar analysis of the Penlac profile could not be undertaken because it follows a non-steady state permeation. The values of Q_{24} h were not significantly different for the transungual patch and Penlac® at 97.39 ± 26.96 µg/cm^2 and 112.52 ± 29.48 µg/cm^2, respectively (p-value > 0.05). It is important to note that the $Q_{24\ h}$ for both the formulations is similar but the process of delivering the drug across the skin is different. The skin permeation profile for Penlac nail lacquer does not follow the Fickian diffusion process, but shows a non-steady state permeation profile. In case of non-steady state permeation the concentration gradient cannot be considered constant (Brodin *et al.*, 2009). Non steady state permeation is seen when the molecule permeates the membrane readily and there is a drop in the drug concentration in the donor compartment with

Figure 2: USP Apparatus 5 for Drug Release Studies on the Transungual Patch

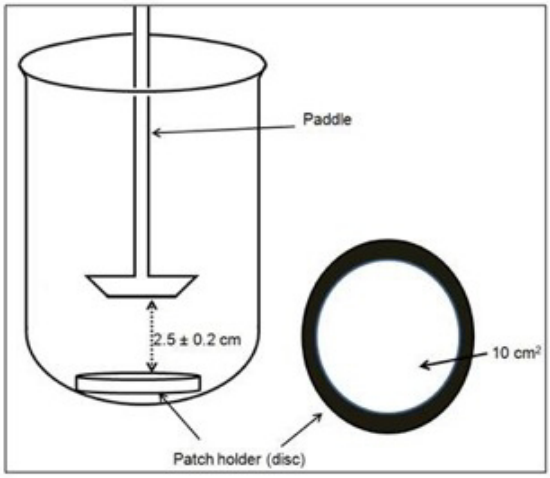


Figure 3: A) Drug Release Profile for CPO from the Transungual Patch B) Cumulative Amounts of CPO Permeating Across the Skin from the Transungual Patch and Penlac® Nail Lacquer C) Concentration of CPO in the Epidermal and Dermal Layers of the Skin, *p-value < 0.05

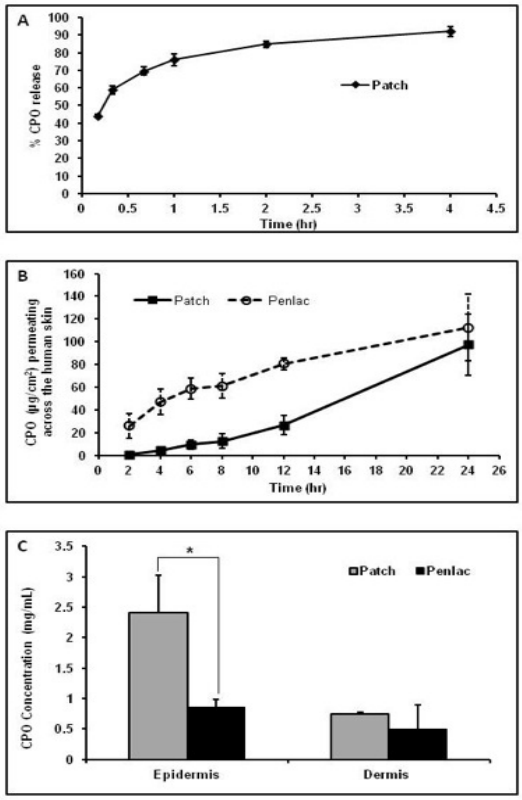
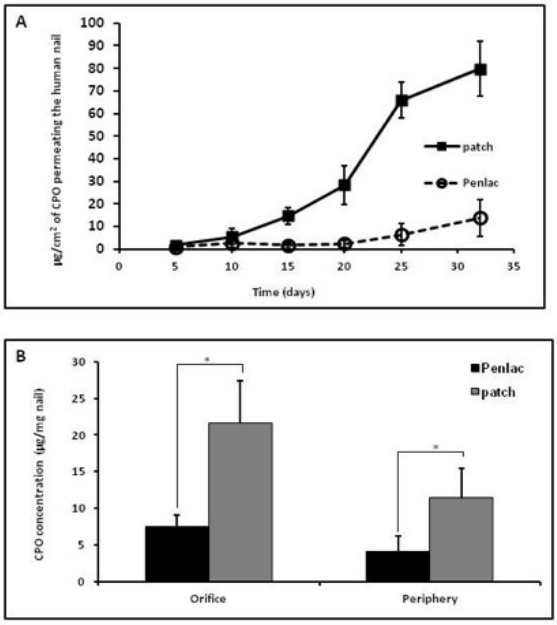


Figure 4: A) Cumulative Amounts of CPO Permeating Across the Human Toenail from the Transungual Patch and Penlac® Nail Lacquer B) Concentration of CPO in the Area of Toenail Treated with the Formulations (orifice) and Area of Toenail Around the Area Treated with the Formulation (periphery), * p-value <0.05



a simultaneous increase in the drug concentration in the receiver compartment (Brodin *et al.*, 2009). After application of the lacquer formulation on the skin, the solvents evaporate and the concentration of ciclopirox in the film increases from 8% to 34.8% (Baran *et al*., 1998; Bohn and Kraemer, 2000). Ethyl acetate is one of the solvents in the nail lacquer. It is also reported to act as a penetration enhancer on the skin (Paudel et al., 2010; Trommer and Neubert, 2006). It is speculated that there is increased permeation of ciclopirox after the application of lacquer, due to the action of ethyl acetate and the high concentration gradient across the skin membrane. This leads to immediate permeation of ciclopirox and a possible decline in concentration of the drug in the donor

compartment resulting in the non-steady state permeation profile. Therefore the cumulative amounts of CPO and CP permeating the skin at end of 24 h ($Q_{24\ hr}$) after application of the transungual patch and Penlac® nail lacquer respectively were compared. The values of $Q_{24\ hr}$ were not significantly different for the transungual patch and Penlac®. The lag-time and the steady state flux for the transdermal permeation of CPO from a previous study using hydro-alcoholic vehicle containing hydroxypropyl chitosan through excised hairless mouse skin was 1.51 ± 0.20 h and 127.45 ± 46.29 µg/cm^2/h, respectively (Monti *et al.*, 2005). This previous study aimed at increasing the transdermal permeation of CPO. The results from the transungual patch show a lower steady state permeation flux of 5.45 ± 1.74 µg/cm^2/h and a longer lag-time of 6.04 ± 1.75 h. Our transungual patch was designed to minimize the transdermal permeation and enhance delivery to the epidermis of the skin. Also, the membrane used in the literature study was hairless mouse skin which tends to over predict human skin permeation. This could explain the increased transdermal permeation of CPO in the study over our transungual patch. The patch formulation consists of 150%w/w of PG. The use of this high concentration of PG is integral part of the fabrication process to keep CPO and TU in solution and impart flexibility to the HPMC matrix. The skin permeation data for the transungual patch indicates that the effect of PG seen in the preformulation screening study (Palliyil *et al.,* 2013) to minimize skin permeation is successfully retained.

In Vitro Skin Accumulation

The thicknesses of the epidermis and the dermis were used to calculate the volume of each of the layers. The volume (cm^3 or mL) was calculated as a product of area of drug exposure and thickness of the skin layers. Figure 3C shows the concentration of CPO within the epidermis and dermis after the skin permeation study for 24 h. The concentration of CPO within the epidermis was significantly higher after treatment with the patch compared to that of Penlac® nail lacquer (p-value <0.05). However, the drug concentrations within the dermis were not significantly different. The enhancement factor, EF_{epi} (Equation 4) for the concentration of CPO attained in the epidermis in presence of the transungual patch was 2.8. Additionally, there was selective accumulation of CPO within the epidermis which had a 3.2 fold higher concentration of CPO than in the dermis upon application of the patch. This shows that the selective accumulation of CPO within the epidermis seen during the preformulation screening of PG (Palliyil *et al.*, 2013) was retained in the transungual patch formulation. In case of Penlac®, the accumulation of CPO within the epidermis was 1.7 fold higher than the dermis. The EF_{der} for the patch formulation was only 1.5. The skin penetration data suggests that the patch formulation will deliver the drug efficiently into the target tissue (epidermis of the nail folds) while limiting its permeation across the skin.

In Vitro Transungual Permeation

The transungual permeation was performed using human cadaver toenails. Figure 4A shows the *in vitro* transungual permeation of CPO across human toenails. The permeation flux attained after 32 days of application of the patch formulation is 4.2 fold higher than that for Penlac® nail lacquer (Table 4). The drug concentration in the orifice and the periphery is shown in Figure 4B. The concentration of CPO attained in both the orifice and the peripheral layers of the toenails were

Table 3: Goodness of Fit of the Three Kinetic Models		
Kinetic Model	**R^2**	**Adjusted R^2**
Zero order	0.9083	0.8625
First order	0.9890	**0.9834**
Square root of time (Higuchi)	0.9617	0.9425

Table 4: Transungual Permeation Parameters			
Formulation	**Permeation Flux ($\mu g/cm^2/day$)**	**Lag-Time (days)**	**$C_{32\ days}$ ($\mu g/mL$)**
Transungual patch	4.10 ± 0.59	11.45 ± 1.25	4.08 ± 0.67
Penlac®	0.98 ± 0.56	18.36 ± 1.20	0.79 ± 0.46

significantly greater than that for Penlac® (p-value < 0.05). The thicknesses of the toenails (0.86 ± 0.053 mm) were used to determine the volume of the nail. This volume was used to calculate the drug concentration within the nail. The CPO concentration within the orifice part of the nail is 14.09 ± 3.67 mg/mL and 5.22 ± 1.7 mg/mL for the patch and Penlac® respectively. The cumulative amount of CPO permeating the human toenails on day 32 (79.69 ± 12.12 µg/cm^2) is comparable to the amount of ciclopirox (free drug) permeating the porcine hoof from acrylate adhesive matrix in presence of penetration enhancers PEG200 (77.4 ± 30.8 µg/cm^2) and Triacetin (73.0 ± 21.4 µg/cm^2) at end of 4 weeks (Myoung and Choi, 2003). Porcine hoof has a lower density of keratin fibers and is therefore more permeable than human nails (Monti *et al.*, 2011). The studies performed using porcine hoof therefore overestimate the drug permeation. The novel patch formulation is therefore better than the reported patches containing ciclopirox (free acid) at delivering CPO across the human nail. The concentration of CPO in the nail for the patch and the lacquer formulations is significantly higher than the nail minimum fungicidal concentration (Nail-MFC) of 16 – 32 µg/mL reported in literature (Schaller *et al.,* 2009).

CONCLUSION

The main aim of the current work was to incorporate previously selected penetration enhancers (TU and PG) into a CPO loaded transungual (nail) patch to develop an efficient transungual drug delivery system when compared with a commercial nail lacquer. A prototype transungual formulation containing the antifungal drug CPO (10% w/w), TU (10% w/w) and PG (150% w/w) of the dry weight of hydrophilic polymer was designed for immediate release of CPO and enhanced permeation into and across the human nail plate. The patch efficiently delivered CPO to the epidermal layer with minimal transdermal permeation in 24 h. Thus, the patch successfully delivered CPO to the two identified target tissues of the human nail (14.09 ± 3.67 mg/mL) and the epidermis of nail folds (2.42 ± 0.60 mg/mL) at concentrations greater than the reported minimum inhibitory concentrations (0.04 to 1.0 µg/mL) of CPO. Thus the novel transungual patch performed more efficiently *in vitro* as a topical delivery system in comparison to a commercial topical formulation (Penlac® nail lacquer). This reformulation strategy for CPO has shown promising results *in vitro* and warrants further *in vivo* studies.

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