A PREFORMULATION DEVELOPMENT STUDY FOR THE TRANSUNGUAL DELIVERY OF THE ANTIFUNGAL DRUG ECONAZOLE NITRATE

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ABSTRACT

Onychomycosis , also known as "dermatophyticonychomycosis," "ringworm of the nail," and "tineaunguium", means fungal infection of the nail. This is the most common disease of the nails and constitutes about a half of all nail abnormalities. This condition may affect toenails or fingernails, but toenail infections are particularly common. The prevalence of onychomycosis is about 6–8% in the adult population. The most common symptom of a fungal nail infection is the nail thickening and discoloration. The Treatment of onychomycosis is challenging because the infection is embedded within the nail. This makes it difficult to reach. The full elimination of symptoms is slow. It may take a year or more, since new nail growth must entirely replace old, infected growth. Onychomycosis is an opportunistic infection in special subpopulations of patients suffering from diabetes, psoriasis, HIV/AIDS etc. Such conditions affect patients physically as well as socially and psychologically and can seriously affect the quality of life. Oral therapy suffers from systemic adverse effects and drug interactions whereas topical therapy is limited by low permeability of the nail plates. By focusing on topical therapy in this study, the use of chemical therapy with penetration enhancers will be evaluated.

Most cases of onychomycosis show infection and inflammation of the nail folds (skin surrounding the nails). Therefore for an efficient treatment of OM, the antifungal drugs must be delivered to two target tissues – human nail and the nail folds. The major challenges in developing a topical formulation for treatment on OM are:

a) Achieving antifungal drug minimum inhibitory concentration (MIC) in the epidermis of the nail folds.

b) Enhancing penetration and permeation of the antifungal drug across the human nail to reach the nail bed and achieve the necessary MIC (tissue underneath the nail).

The objectives of this project were to: 1) To determine the critical factors affecting the delivery of econazole nitrate across the human nail, 2) To screen and select the optimal penetration enhancer(s) specific for econazole nitrate delivery into the target tissue(s). 3) To study how different pH affects tissue penetration.

Ten penetration enhancers were screened for their ability to improve solubility, in vitro nail penetration, in vitro skin permeation, and in vitro skin penetration of the antifungal drug econazole nitrate. In contrast to transdermal drug delivery, the main selection criteria for skin penetration enhancer in topical drug delivery were increased drug accumulation in the epidermis and minimal permeation across the skin. Thiourea improved the solubility and nail penetration of econazole nitrate. It also produced enhancement in the transungual diffusion of the drug. It was the selected nail permeation enhancer and the selected skin penetration enhancer of econazole nitrate. In different the pH study, pH5 PBS buffer was the most effective pH for both enhancing the drug amount in the nail and decreasing the keratin binding. This increases the free drug amount accumulated in the target tissues.

The proposed preformulation strategy helps to select appropriate enhancers for optimum topical delivery and paves way towards an efficient topical formulation for passive transungual drug delivery.

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INTRODUCTION

1.1 Abbreviations

EN: Econazole Nitrate

TU: Thiurea

OM: Onychomycosis

MIC: minimum inhibitory concentration

DLSO: Distal and Lateral SubungualOnychomycosis

EO: EndonyxOnychomycosis

OM: Onychomycosis

SO: Proximal SubungualOnychomycosis

1.2 Introduction

The nails serve several important functions. When they are infected by fungal organisms, these functions are severely impaired. In addition, the quality of life, in terms of self-esteem and social interaction, is adversely affected when fungal nail infections are present. Finally, when finger and/or toenails are abnormal this may interfere with the patients' occupation. Onychomycosis is the most common nail disease, responsible for approximately 50% of nail problems. In addition, onychomycosis may be responsible for up to 10% of all cutaneous and appendageal disorders(R.K.SCHER,1994). Onychomycosis has significant social, psychologic, health, and occupational effects. Relevance of quality-of-life issues to overall health, earning potential, and social functioning should prompt reconsideration of the value of aggressive treatment of and financial coverage for onychomycosis (*J Am AcadDermatol 1998*).

1.3 Onychomycosis

1.3.1 Definition, Epidemiology and Risk Factors

"Onychomycosis" traditionally referred to a non-dermatophytic infection of the nail but is now used as a general term to denote any fungal nail infection (tineaunguium specifically describes a dermatophytic invasion of the nail plate). It is caused by dermatophytes, yeasts and molds.

1.3.2 Pathogens in onychomycosis

The fungi that cause onychomycosis are the dermatophytes, yeasts and molds. Studies of sampled material from infected nails have identified that over90% of onychomycoses are caused by two dermatophytes: *Trichophytonrubrum* (71% of all infections) and *Trichophytonmentagrophytes* (20%) (Fig. 1.1). The pathological distribution of the different pathogens is not even. Rather, it depends on various factors such as geography, climate and migration.

Among the yeasts, the most commonly implicatedorganism in onychomycosis is *Candida albicans*. Although yeast infections most commonly affect the fingernails, C. albicans infections may also account for approximately 10% of cases of toenail onychomycosis. Saprophytic molds, particularly Scopulariopsis brevicaulis, can be isolated from approximately 11% of cases
of toenail onychomycosis.

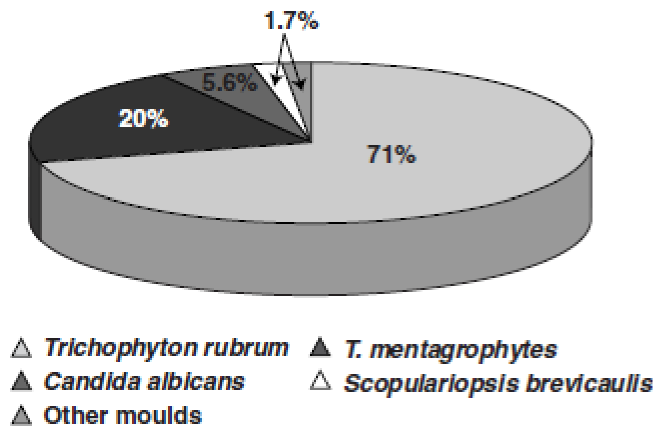


Figure 1.1 The fungi involved in onychomycosis.

1.3.3 Classification of Onychomycosis

There are five major classic types of onychomycosis, most of which can be further subdivided:

1) Distal subungual onychomycosis: can be further subdivided into distal lateral subungal onychomycosis and endonyxsubungal onychomycosis. Distal lateral subungal infection, the most common form of the disease, begins on the distal section of the nail and spreads under the nail, infecting the nail bed.
2) White superficial onychomycosis (WSO): is more commonly associated with T.mentagrophytes var. interdigitale than with T. rubrum infections. However, it is relatively simple to obtain a sample of the fungi responsible for infection. Superficial onychomycosisinfections are relatively simple to treat and respond well to topical therapy.
3) Proximal subungualonychomycosis: is fungal penetration of the newly formed nail plate through the proximal nail fold. It is the least common form of tinea unguium in healthy people, but is found more commonly when the patient is immune-compromised.

4) Candidal onychomycosis: is Candida species invasion of the fingernails, usually occurring in persons who frequently immerse their hands in water. This normally requires the prior damage of the nail by infection or trauma.
5) Total Dystrophic Onychomycosis: Total dystrophic onychomycosis is used to describe end-stage nail disease, although some clinicians consider it a distinct subtype. It may be the end result of any of the four main patterns of onychomycosis. The entire nail unit becomes thick and dystrophic.

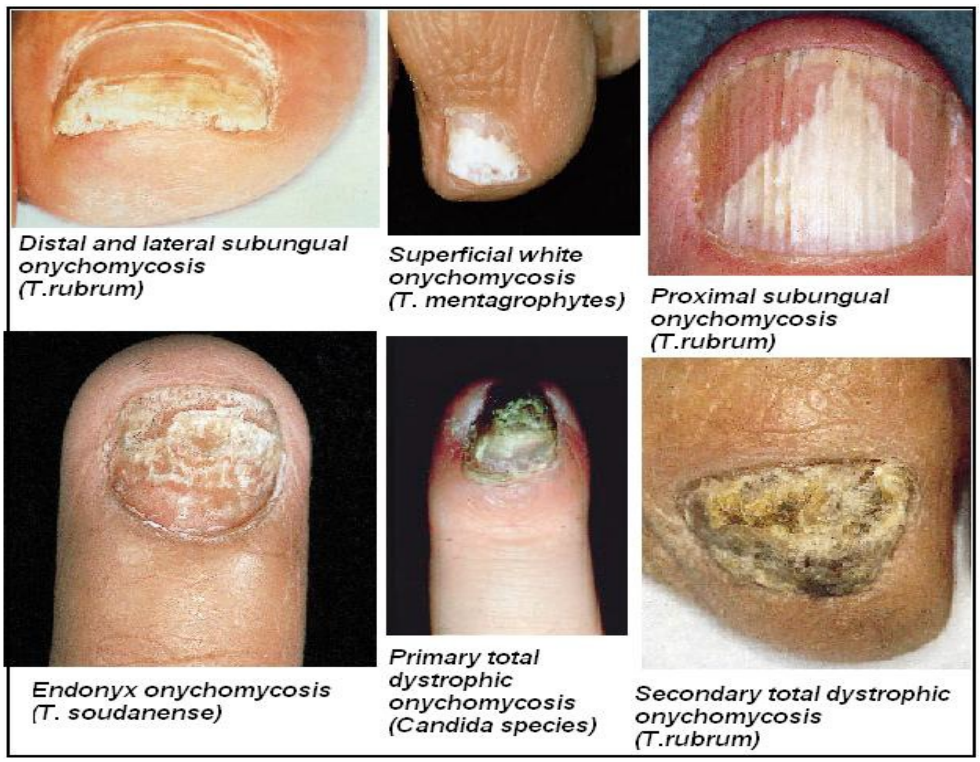


Figure 1.2: Types of Onychomycosis (Baran R et al 1998 and Schlefman BS 1999).

1.3.4 Impact of Onychomycosis

Onychomycosis impairs normal nail functions, causes considerable pain, interferes with daily activities, and has negative psychosocial effects. According to a study conducted by a total of 258 patients with confirmed onychomycosis were surveyed by telephone at three centers. Responses to a standardized quality-of-life questionnaire were analyzed for patient demographics, physical and functional impact, psychosocial impact, and economic impact. Highest positive responses were nail-trimming problems (76%), embarrassment (74%), pain (48%), nail pressure (40%), and discomfort wearing shoes (38%). Ability to pick up small objects was impaired in 41% of subjects with fingernail involvement. More than 58 onychomycosis-related sick days and 468 medical visits (1.8 per subject) were reported during a 6-month period(*J Am AcadDermatol 1998*).Furthermore, patients who have onychomycosis may develop psychological disorders related to concern about the abnormal appearance of their nails. Fungal nail infections may also lead to the spread of organisms to adjacent and distant areas of the skin. This leads to further impairment of the quality of life, with mental and social implications leading to reduced self-esteem and limitation of interaction with others.

1.3.5 Diagonosis of Onychomycosis

The clinical presentation of dystrophic nails should alert the clinician to the possibility of onychomycosis; however, because fungi cause only about half of all nail dystrophies, the use of appropriate diagnostic techniques including direct microscopy and fungal culture is important to ensure correct diagnosis and treatment. The clinical appearance of the nail and the patient's history will help differentiate fungal from non-fungal etiologies of nail dystrophies (*BONI E. ELEWSKI , July,1998*).

1.3.6 Challenges in treatment of OM

The following are the challenges currently faced in effective treatment of OM:

1) Frequent relapse or reinfection: Recurrence of OM ranges from 10-53% (*Scher R K et al 2007; Sigurgeirsson, B. et al 2002; De Doncker, P.D. et al 1997 and Villars, V.V. and Jones, T.C. 1992*). Recurrence entails a case where the nail apparatus is devoid of the clinical symptoms but mycological cure was not attained or when a new infection develops during or after termination of treatment. This often occurs

2) Slower growth rate of nails: The healthy fingernails and toenails grow at the rate of 3.47 mm/month and 1.62 mm/month respectively (*Yaemsiri, S. et al 2010). This means that the fingernail requires 4 to 6 months to completely grow out and for a toenail this period is 12-18 months (Geyer, A.S. et al 2004*). The nails that are severely infected with OM show a relatively slower growth compared to healthy nails. The growth rate also diminishes by 0.5 % per year after 25 years of age (*Geyer, A.S. et al 2004*).

3) Increasing population of predisposed patient population

4) Lack of antifungal drugs designed specifically for OM which have increased spectrum of action, decreased drug-drug interactions, decreased relapse and reinfection.

5) Low patient compliance due to longer duration of therapy (*Gupta, A.K. and Simpson, F.C. 2012*)

1.6 Econazole Nitrate

Econazole nitrate is an antifungal medication of the imidazole class. For local application the nitrate salt of econazole is used. In preliminary investigations of systemic use in a few patients econazole base has been administered orally or intravenously. In uncontrolled studies in large numbers of patients, econazole nitrate has been administered topically in the treatment of dermatomycoses because it is effective against a wide variety of fungi. It has also been used vaginally in the treatment of vaginal candidosis. In dermatological studies about 90% of patients were cured, often after a relatively short treatment period(2 to 6 weeks). (R.C.Heel, R.N.Brogden, 1978)

1.6.1 Physicochemical Properties

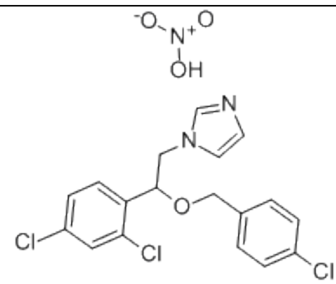
Molecular weight	444.7 g/mol
Molecular formula	$C_{18}H_{16}Cl_3N_3O_4$
Chemical name	1-(2,4-dichloro-beta-((p-chlorobenzyl)oxy)phenethyl)imidazole nitrate
Log P	5.5
pKa	6.77
Melting Point	162°C
Water Solubility	<0.1 g/100 mL at 19 ºC
Description	White crystalline powder
Chemical Structure	

Figure 1.6 White crystal form of Econazole nitrate

1.6.2 Mechanism of Action

Econazole interacts with 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Econazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis.

1.6.3 Pharmacodynamic properties

- Antifungal Activity

In a typical report of its in vitro antifungal activity, econazole and in some cases references substances were tested against various microbes using the serial decimal dilution test in liquid media, giving final drug concentration in the culture media from 0.001 to 1000 µg/ml. Against dermatophytes, econazole was usually more active than the antifungal agent tolnaftate, the minimum inhibitory concentration (MIC) of econazole for complete inhibition of species of *Microsporum* ranging from 0.1 to 1.0µg/ml. When tested against yeasts, econazole was more active than nystatin against *Torulopsisglabrata, Ctyptocodcusneoformans* and some species of Candida, while both agents were equiactive against *Candida albicans.* Noncomparative *in vitro* tests have also shown activity against various other molds, actinomycetes and other fungi, the most common of which are included in the table below.

Table 1.1: The in vitro Minimum Inhibitory Concentrations (MICs) of EN against different strains of dermatophytes and yeasts

Species [1]	Inhibitory concentration (µg/ml)	
	econazole	reference agent [2]
Dermatophytes		
Microsporumcanis[3]	0.1-1.0	10-100
Trichophytonmentagrophytes [3]	0.01	10
Trichophytonrubrum[3]	0.1-1.0	0.1
Trichophytoninterdigitale	1.0	1000
Yeasts		
Saccharomyces cerevisiae	100	10
Torulopsisglabrata	100	10
Cryptacoccusneoformans	10	1.0
Candida albicans	100	10
Candida tropicalls	100	10
Candida pseudotropicalls	10	1.0
Candida stellatoidea	10	- [4]
Candida krusei	100	1.0
Candida parapsilosis	10	-

Note:

1. Econazole has shown *in vitro* activity against a number of other, less common fungi in addition to those listed here.
2. Reference agent in tests against dermatophytes = tolnaftate; in tests against yeasts = nystatin.
3. More than 1 strain or phase of growth tested.
4. A dash indicates no pronounced inhibition at lower concentration.

• Antibacterial Activity

Econazole, like miconazole has *in vitro* activity against some Gram-positive cocci and bacilli, the minimum inhibitory concentrations for these organisms being similar to those for yeasts, but it does not appear to have useful activity against Gram-negative organisms.

Table 1.2: The Minimum Inhibitory Concentration (MIC) of EN against bacteria

Bacteria	MIC (µg/ml)			
	econazole		miconazole	
	base	nitrate	base	nitrate
Staphylococcus aureus	12.5	12.5	6.25	6.25
Staphylococcus epidermidis	12.5	12.5	6.25	6.25
Streptococcus pyogenes	12.5	12.5	6.25	6.25
Streptococcus bovis	6.25	6.25	3.12	3.12
Streptococcus faecalis	25	25	25	25
Streptococcus faecium	25	25	25	25
Corynebacterium diphtheria	0.78	0.78	0.39	0.39
Listeria monocytogenes	1.56	1.56	1.56	1.56
Bacillus subtilis	12.5	12.5	3.12	3.12

1.6.4 Pharmacokinetics

From the patients tested in such PK studies it appears that about 90% of topically applied econazole nitrate (as the cream) remain on the skin surface, occlusion only slightly increasing the extent of absorption. Topical administration of econazole provides little systemic absorption. Following administration of econazole vaginal cream, less than 3% of the dose is recovered in urine and feces over 96 hours. The plasma protein binding of econazole is greater than 98%. The volume-of-distribution and plasma clearance of econazole have not been reported.

The elimination half-life of econazole ranged from 4 to 6 hours in two patients with normal renal and hepatic function. Following administration of radio labeled econazole, 39% to 40% of the dose is excreted into the urine and 23% to 31% is recovered in the feces (probably as metabolites) over 5 days. No unchanged econazole is detected in the urine. There are no data on the pharmacokinetic disposition of econazole topical in patients with renal and/or liver dysfunction. There are no data on the hemodialysis or peritoneal dialysis clearance of econazole topical.

1.6.5 Chromatographic analysis of EN

The resulting solution was filtered and analyzed using HPLC. Acetonitrile: Water (1:1) was used as the mobile phase with a flow rate of 1 mL/min in a C18 column. 0.5% formic acid and 0.1% triethylamine were added into the mobile phase in order to get better shape of the curve and also prevent tailing issue. The wavelength of 230nm was used for detection of EN. Any specific alteration in this method will be described in the corresponding chapters. Figure 1.7 shows a representative chromatogram for EN.

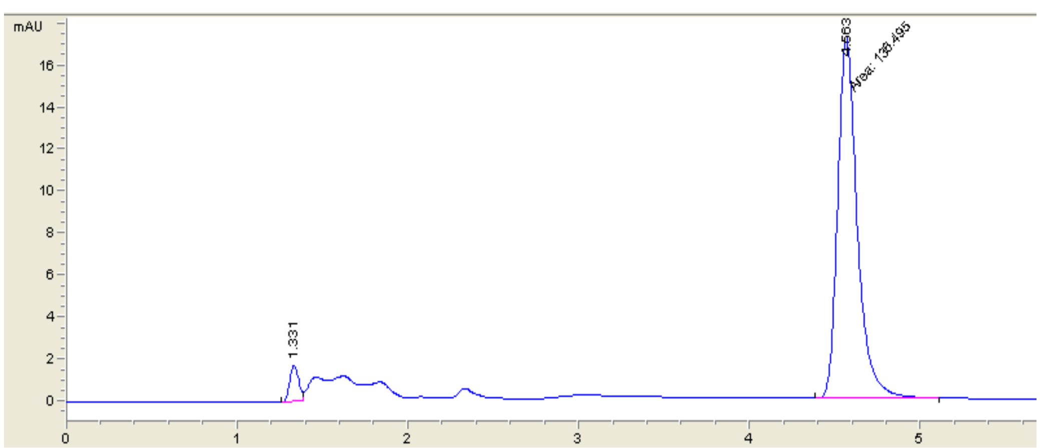


Figure 1.7: HPLC Chromatogram of EN

1.6.6 Rationale for selection of EN

Econazole nitrate is very slightly soluble in water and soluble in methanol. It has a low molecular weight which will help it permeate to the target tissues. It has a log P of 5.5, which means it has higher lipophilicity to get higher partitioning to biological membranes but inhibit permeation across biological membranes (target tissues). Future the experiments into a patch, with the hydrophobic property make econazole easy to combine with polymer matrixes. Compared to ciclopirox which is soluble in water, it has a balanced hydrophilic and hydrophobic property. Itroconazolean ketoconazole also have low solubility but their aqueous solubility make their feasibility questionable. Therefore, econazole is the best balanced drug which still have reasonable aqueous solubility and high partition coefficient.

Econozole is fungistatic and fungicidal with a broad spectrum of action. Due to its antibacterial effects EN will be effective against secondary bacterial infection associated with OM. According to the preliminary study, it is stable in the normal conditions and will not have any chemical interactions with penetration enhancers.

After topical application to the skin of normal subjects, systemic absorption of econazole nitrate is extremely low. Although most of the applied drug remains on the skin surface, drug concentrations were found in the stratum corneum which, by far, exceeded the minimum inhibitory concentration for dermatophytes. Inhibitory concentrations were achieved in the epidermis and as deep as the middle region of the dermis. Less than 1% of the applied dose was recovered in the urine and feces. Econazole is approved by the FDA for the treatment of tineapedis, tineacruris, tineacorporis, tineaversicolor, and cutaneous candidiasis and other fungal infection diseases.

1.4 Tissues affected by Onychoycosis

1.4.1 Human Nail

The nail unit (schematically shown in Figure 1.3) is composed of the nail plate, nail folds, nail matrix, nail bed, and the hyponychium. The nail matrix forms the nail plate whose growth rate varies considerably among individuals; average values of 3 mm per month (fingernails) and 1 mm per month (toenails) are often used. Thus, on average, a normal fingernail grows out completely in about 6 months while a normal toenail in about 12 – 18 months. The most obvious part of the nail unit – the nail plate – is a thin (0.25 – 0.6 mm), hard, yet slightly elastic, translucent, convex structure and is made up of approximately 80 – 90 layers of dead, keratinized, flattened cells which are tightly bound to one another. The cells at the dorsal surface of the plate overlap (Figure 1.3A), producing a smooth (and poorly permeable) surface, and a cross-section view highlights the plate's compact nature (Figure 1.3B). The nail plate can be divided into two layers: dorsal and ventral, based on differential ultrasound transmission. The ventral layer is more plastic, while the dorsal one is hard and brittle. Some authors consider the nail plate to consist of three strata: dorsal, intermediate and ventral, the latter being taken as the few layers of cells, which connect the nail plate to the nail bed. Chemically, the nail plate consists mainly of proteins, namely low sulphur keratins, and high sulphur and high glycine/tyrosine proteins. The latter two groups are believed to form an inter filamentous matrix, while the low sulphur keratins form 10 nm filaments. The plate also contains water at 10 – 30%, and very small amounts of lipid at 0.1 – 1%.

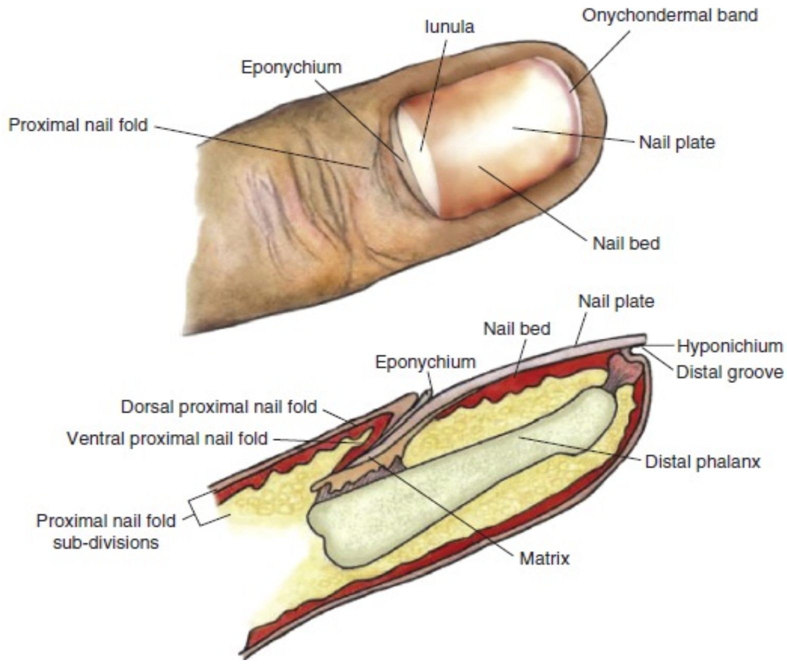


Figure 1.3: Components of the nail apparatus (Murdan S. 2008)

Figure 1.4: Cadaver toenails obtained from Anatomy Gifts Registry: Healthy Cadaver toenails (left panel) and Infected Cadaver toenails (right panel)

1.4.2 Human Skin

The skin can be considered to have three distinct layers of tissue.(*Trop J PharmRes, April 2009*)

1. Non-viable epidermis (stratum corneum)

2. Viable dermis

3. Subcutaneous connective tissue (hypodermis)

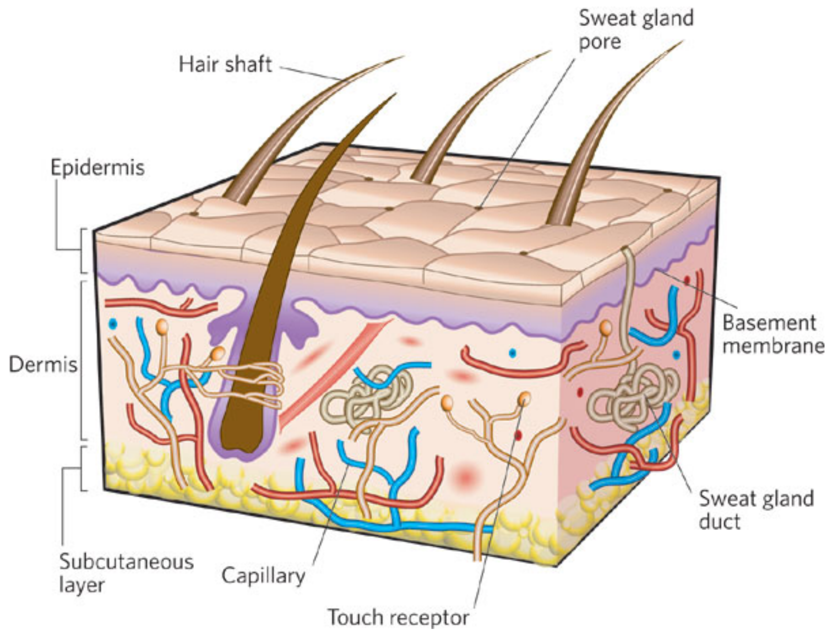


Figure 1.5: Layers of the skin

Stratum corneum is the outer most layer of skin. This is the actual physical barrier to most substance that come in contact with the skin. The stratum corneum is 10 to 20 cell layers thick over most of the body. It consists of lipid (5-15%) including phospholipids, glycosphingolipid, cholesterol sulfate and neutral lipid. The protein (75-85%) is mainly keratin.

Beneath the epidermis is the dermis. It is a structural fibrin which has a thickness range from2000 to 3000 µm and consists of a matrix of loose connective tissue composed of fibrous protein embedded in an amorphous ground substance. The subcutaneous tissue is not actually considered a true part of the structured connective tissue is composed of loose textured, white, fibrous connective tissue containing blood and lymph vessels, secretory pores of the sweat gland and cutaneous nerves. Most investigators beleivethe drug permeates through the skin and enters the circulatory system before reaching thehypodermis. Although, the fatty tissue could serve as a depot of the drug.

Permeation of skin can occur by diffusion via:

1. Transdermal permeation, through the stratum corneum.
2. Intercellular permeation, through the stratum corneum.
3. Transappendaged permeation, via the hair follicle, sebaceous and sweat glands.

1.5 Treatments for Onychomycosis

Both topical and oral agents are available for the treatment of fungal nail infection. The primary aim of treatment is to eradicate the organism as demonstrated by microscopy and culture*(British Journal of Dermatology 2003).*

1.5.1Topical treatment

Topical drug delivery is especially suitable for Onychomycosis (2 – 13%) and nail psoriasis (1 - 3%), which affect general population, and make up the bulk of nail disorders. Topical therapy avoids the adverse events and drug interactions of the systemic antifungal agents. It is non-invasive when compared to the injection antipsoriatic agents are injected into the nail folds. Target sites for the treatment of onychomycosis and other nail disorders resides in the nail plate, nail bed and nail matrix. Various topical therapies for nail disorders, which have been studied so far, are: Lacquers, Gels / Solutions, Creams / Pastes, Colloidal systems / Liposomes, Powders, Aerosols /Foams /Sprays.

A Bandage was developed. This consists of a T-shaped adhesive backing and a flexible pad having animpervious backing with a nail-shaped cavity (containing active solute along with other sedatives) *(T Praveen Kumar et.al, IJPRR 2013)*.

Table 1.3: Developed Formulations For Nail Disorders

Sl.No	Name of the product	Name of Drug	Uses/Indications	Name of Company
1	Eco-Nail nail lacquer	5% econazole+18% SEPA nail lacquer	Promotes the release of econazole from dried lacquer film, creating a large chemical gradient at the lacquer nail interface, to drive econazole into the deep nail plate. SEPA acts as a percutaneous penetration enhancer.	MacroChem Corporation
2	Loceryl nail film	Antifungal drug Amorolfine	A non-water-soluble film of Amorolfine formed on the nail plate, and this film remains in place for 1 week. It contains a high concentration of drug and forms a depot from which the drug is delivered and allows the drug to permeate the nail plate.	Galderma Australia Pty Ltd
3	UmectanailFilm	Urea 40%	Psoriatic nails, brittle and thick nail, and Calluses	JSJ Pharmaceuticals Allergan Inc.
4	Tazorac 0.1% Gel	Tazarotene	Used in the Treatment of Fingernail Psoriasis	Labtec
5	Zalain nail Patch	Sertaconazol Nitrate	Once-a-week nail patch for treatment of onychomycosis&onychodystrophy	Dermik
6	Penlac nail lacquer	Ciclopirox topical solution	A broad-spectrum antifungal medication that also has antibacterial and anti-inflammatory properties	Laboratories Inc.

1.5.2 System treatment

The three drugs currently licensed for general use in onychomycosis are listed in Table 1.2. The two other systemic agents available for oral use, ketoconazole and fluconazole, are not licensed for nail infection. Ketoconazole may be used in some recalcitrant cases of yeast infection affecting the nails but cannot be prescribed for dermatophyte onychomycosis because of problems with hepatotoxicity. The use of fluconazole thus far has concentrated on vaginal candidiasis and systemic yeast infections although it is active against dermatophytes. There are some published studies of its use in nail infection but the dose and duration of treatment are not yet clear and it is not licensed for this indication in the U.K., nor does it appear likely to be so in the near future(*2003 British Association of Dermatologists, British Journal of Dermatolog*).

Table 1.4. Systemic agents for onychomycosis, with major advantages and disadvantages, and strength of recommendation and quality of evidence grading

Drug	Advantages	Disadvantages	Main drug interactions	Strength of recommend
Griseofulvin	Licensed in both adults and children, inexpensive, extensive experience	Lengthy treatment necessary in both fingernail and toenail infection; poor cure rates; high relapse rates; no paediatric formulation currently available; contraindicated in lupus erythematosus, porthyria and severe liver disease	Warfarin, ciclosporin. Oral contraceptive pill	*B-I*
Terbinafine	Fungicidal; high cure rates(compared with griseofulvin); short duration of therapy; good compliance	No U.K licence for children; no suspension formulation; idiosyncratic liver and skin reactions; reversible taste disturbance in 1:400 patients	Plasma concentrations reduced by rifampicin, increased by cimetidine	A-I
Itraconazole	Active against Candida albicans; pulsed treatment regimens are possible	Less effective in dermatophyte onychomycosis than terbinafine; monitoring of liver function required for treatment durations of longer than 1 month; not licensed for use in children and contraindicated in pregnancy	Enhanced toxicity of anticoagulants(warfarin),antihis tamines (terfenadine and astemizole),antipsychotics (sertindole),anxiolytics(midazol am), digoxin, cisapride, ciclosporin and simvastatin (increased risk of myopathy); reduced efficacy of itraconazole with concomitant use of H2 blockers, phenytoin and rifampicin	A-I

1.5.3 Summary of conclusions

1). Treatment should not be commenced before mycological confirmation of infection.

2). Dermatophytes are by far the commonest causal organisms.

3). Culture of yeasts and non-dermatophyte molds should be interpreted carefully in each individual case. In the majority, yeasts are likely to be a secondary infection and non-dermatophyte molds to be saprophytic in previously damaged nails.

4). Topical treatment is inferior to systemic therapy in all but a small number of cases of very distal infection or in SWO.

5). Terbinafine is superior to itraconazole both in vitro and in vivo for dermatophyte onychomycosis, and should be considered first-line treatment, with itraconazole as the next best alternative.

6). Cure rates of 80–90% for fingernail infection and 70–80% for toenail infection can be expected. In cases of treatment failure the reasons for such failure should be carefully considered. In such cases either an alternative drug or nail removal in combination with a further course of therapy to cover the period of regrowth should be considered.

1.7 Strategies to enhance topical delivery of drugs into the nail

The nail barrier is one of the toughest obstacles to drug permeation into the target tissues. For effective topical therapy, ungual drug permeation must be enhanced. This can be achieved by using mechanical, physical or chemical methods, which are discussed below.

1.7.1 Mechanical methods

1.7.1.1 Nail avulsion

Removal of the entire nail plate or partial removal of the affected nail plate is done surgically by total nail avulsion and partial nail avulsion and under local anaesthesia. It is used in clinical studies prior to topical treatment of onychomycosis. (T Praveen Kumar et.al, IJPRR 2013)

1.7.1.2 Nail abrasion

Nail abrasion, using sandpaper nail files is performed prior to antifungal nail lacquer treatment to decrease the critical fungal mass. Nail abrasion involves sanding of the nail plate to reduce thickness or destroy it completely. Sandpaper number 150 or 180 can be utilized. (T Praveen Kumar et.al, IJPRR 2013)

1.7.2 Physical methods

Physical permeation enhancement may be superior in delivering hydrophilic and macromolecular agents.

1.7.2.1 Etching of the nail surface with acid

A brief application (up to 90 s) of a 20% tartaric acid solution or a 10% phosphoric acid gel onto the dorsal surface of nail clippings was used in vitro to modify the nail plate surface prior to application of drug formulations, such as adhesive polymeric films.(Repka MA, Mididoddi PK,2004)

1.7.2.2 Ablation of the nail plate using pulsed lasers

Neevet al. investigated the effects of laser light on nail plates in vitro. Following topical application, laser energy would be absorbed by water or protein in the nail plate and the scattered heat would lead to vaporization (thus, removal) of nail layers. The optimal laser system was found to be the ultra short pulse laser due to its high efficiency, negligible collateral damage. (Neev J, Nelson JS, Critelli M, et al.1997)

1.7.2.3 Micro-needle

It is an enhanced delivery system. This method involves using arrays of microscopic needles to open pores in the SC directly to the skin capillaries, thus facilitating drug permeation. It also has the advantage of being too short to stimulate the pain fibers.

1.7.2.4 Iontophoresis

Iontophoresis involves the application of electric field for the delivery of a compound across a membrane. Drug diffusion through the hydrated keratin of a nail may be enhanced by Iontophoresis. Factors that contribute to this enhancement include electro repulsion/electrophoresis- interaction between the electric field and the charge of the ionic drug; electro osmosis- convective solvent flow in pre-existing and newly created charged pathways; and permeabilization / electroporation- electric field-induced pore induction. Compared to passive transport, Iontophoresis significantly enhanced drug penetration through the nail. (Murthy SN, Wiskirchen DE,2007)

1.7.3 Chemical methods

Use of chemical enhancers has a number of advantages over other methods:

- Ease of drug administration because the enhancer can be incorporated in the formulation.
- It does not require skill for application. This is contrary to some physical methods like drilling and micro-needle.
- Compared to mechanical methods, will not cause any painful sensation and is not invasive.

- Greater activity as entire nail plate can be exposed to the action of chemical enhancers as opposed to physical methods like laser.
- Cheaper than physical methods.

1.8 Penetration enhancers

The low penetration of drugs into the skin and the nail is the primary reason for poor efficacy of topically applied drugs. The use of penetration enhancers is an effective strategy to improve the passive diffusion of the drug into the nail and the skin. Penetration enhancers (PEs) are chemical compounds which interact with the tissue and increase the diffusion of drug molecules through the biological membranes. The properties of an ideal penetration enhancer are summarized below (Williams, A.C. and Barry, B.W., 2004).

- Non-toxic, non-irritant, non-allergenic
- Upon application, the enhancer should work promptly with a predictable and reproducible duration of activity
- Pharmacologically inert
- Should prevent loss of endogenous substances from the body
- Should show reversible change in the barrier properties of the membrane
- Compatible with the active ingredient and other excipients in the formulation
- Comfortable for the patient

During *in vitro* permeation experiments, the membrane is placed between a donor and receiver compartments, where the receiver compartment is maintained under sink conditions for the duration of the study. If the cross sectional area of the membrane is A and its thickness is l, Fick's first law of diffusion can be written as follows (Sinko, P.J. 2006).

$$J = \frac{dM}{Adt} = D\left(\frac{C1-C2}{t}\right) \qquad (1.1)$$

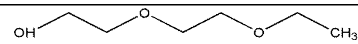
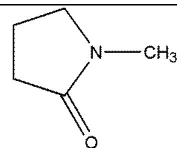
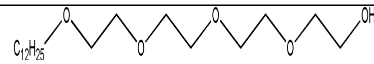
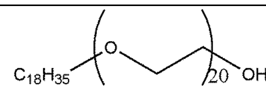
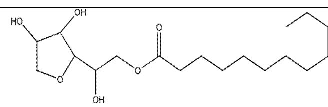
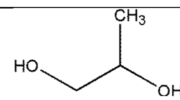
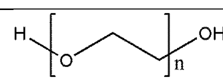
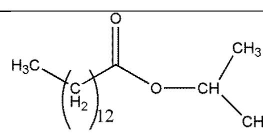
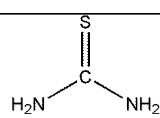
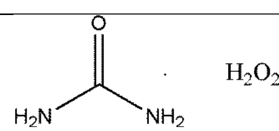
Where, J= steady state flux, D= diffusion coefficient of the drug (cm2/sec), M = amount of drug, t = unit time, C1= Concentration of the drug in the membrane in contact with the donor solution, C2 = Concentration of the drug in the membrane in contact with the receiver solution.

Penetration enhancers may act by one or more of three main mechanisms:

1. Disruption of the highly ordered structure of stratum corneum lipid.
2. Interaction with intercellular protein.
3. Improved partition of the drug, co-enhancer or solvent into the stratum corneum.

In table 1.6, it showed the penetration enhancers been screened in the current project and its mechanism of action.

Table 1.5: Penetration enhancers screened in the current project

Penetration Enhancers (PEs)	**Abbrev**	**Chemical Structure**	**Concentration**	**Mechanism of Action**
Diethylene glycol monoethyl ether or(Transcutol®)	TCL		100%	*Increase drug solubility*
N-methyl pyrrolidone	NMP		10%	*Altering membrane solvent nature, increase drug partition into skin*
Poly(oxyethylene) (4) lauryl ether or BRIJ 30	B30		2.5%	*Potential to solubilize lipids in stratum corneum of skin, increase solubility of drug, emulsify sebum*
Polyoxyethylene (20) Oleyl Ether or BRIJ 98	B98		2.5%	
Sorbitanlaurate or SPAN 20	S20		100%	
Propylene glycol	PG		10%	*PG and PEG 400 shown the 'solvent drag' effect within the skin, solubilizes α keratin*
Polyethylene glycol 400	PEG		100%	
Isopropyl myristate	IPM		100%	*Penetrates into lipid bilayers and increases lipid fluidity*
Thiourea	TU		10%	*Reduces disulpHide linkages in keratin of nail*
Urea-hydrogen peroxide	UH		10%	*Oxidizes disulpHide linkages in nail*

1.9 Objectives of the project

Transungual therapy is considered to be highly desirable to treat nail disorders due to its localized effects and improved adherence. This results in minimal adverse systemic events. However, the effectiveness of topical therapies is limited due to minimal drug permeability through the nail plate. Nail permeability is quite low and limits topical therapy to early/mild disease states. Hence the absorption of drugs into the nail unit, to the nail plate, is highly desirable to treat nail disorders. The nail plate behaves like a concentrated hydrogel to permeating molecules. The diffusion of molecules through the nail plate has been compared to the diffusion of non-electrolytes through polymer gels *(T Praveen Kumar et.al, IJPRR 2013)*. The formulation development of an effective topical therapy of OM is challenging due the barrier properties of the nail, compact structure of the nail plate, location of the infection within the layers of the nail plate and thickness of the nail *(Hui, X. et al 2004)*. Econazole nitrate has already been formulated into the product Eco-Nail lacquer and get into the market by company MacroChem Corporation, it has relatively low MIC and balanced LogP and pKa which make it an optimal choice. In the current project, ten penetration enhancers will be screened. The objective of this was to find the PE which produced the best EN permeation. Additionally, the effect of pH will be investigated. EN is a weak base. It has a positive charge at a pH below 6. The pH is expected to affect both the solubility and partitioning of the drug into the nail.

It is well reported in literature that penetration of a drug into the nail is affected by number of factors, namely: the physicochemical properties of a drug molecule (size, shape, charge, and hydrophobicity), the nature of the formulation (nature of the vehicle and drug concentration), presence of enhancers, nail properties (thickness and hydration), and the interactions between the drug and the membrane (Elkeeb, R. *et al* 2010). Understanding the roles of different interactions between the drug and the target tissues (nail and nail folds) and the impact of selected enhancers on these interactions will help in development of an effective transungual formulation for treatment of the disease.

1.9.1 Hypothesis

“The antifungal drugEconazole nitrate can successfully penetrate the human skin and nail when preformulated with the appropriately selected penetration enhancer(s) in proper pH.”

1.9.2 Specific aims

1) To determine the critical factors to the in vitro transungual delivery of econazole nitrate.
2) To screen and select penetration enhancer(s) specific for econazole nitrate delivery into the target tissue(s).
3) To study how pH will affect the seleted penetration enhancers combined with EN.

CHAPTER 2

THE SELECTION OF THE PENETRATION ENHANCERS:

A PREFORMULATION STRATEGY

2.1 Abbreviation

B30: Brij 30

B98: Brij 98

EN: Econazole nitrate

IPM: Isopropyl myristate

NMP: N-methyl pyrrolidone

PEs: Penetration enhancers

PEG 400: Polyethyleneglycol 400

PG: Propyleneglycol

pH 7.4 PBS : 10 mMPhosphateBuffered Saline pH 7.4

S20: Span 20

TCL: Transcutol

TU: Thiourea

UH: Urea-hydrogenperoxide

2.2 Introduction

The object of this study is the topical delivery of antifungal drug into the target tissues. One of the approaches to increase passive permeation is the use of chemical penetration enhancers (PEs). It was hypothesized that drug delivery into the two target tissues –the human nail plate and the nail folds (skin) will increase with the use of specific PEs. This will subsequently increase the antifungal activity of the resulting topical formulation. The main objective of the study was to screen the PEs (Table 1.6) and select suitable enhancers for delivery of the antifungal drug EN into the nail and nail folds. Figure 2.1 shows the proposed preformulation screening strategy for selection of PEs for transungual and epidermal drug delivery.

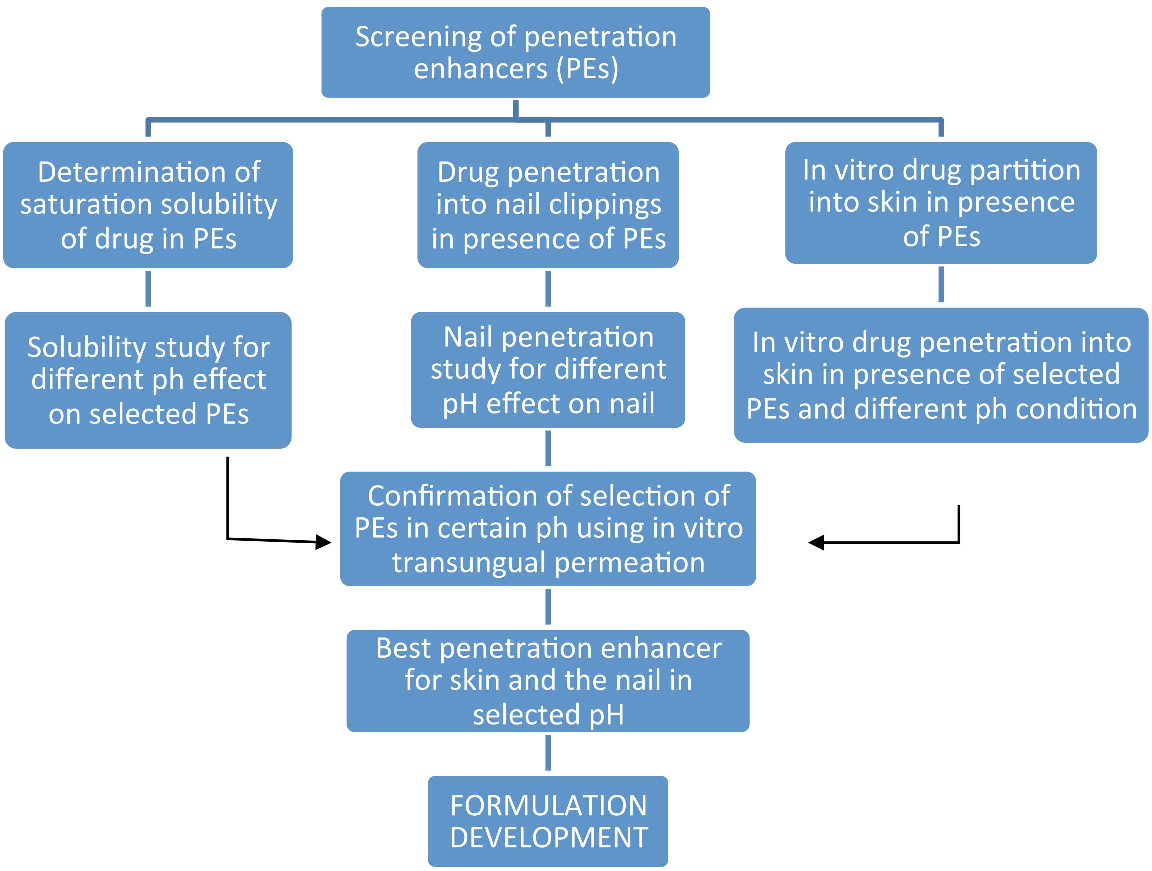


Figure 2.1: Preformulation Strategy for selection of penetration enhancers

2.3 Materials

Econazole nitrate (Spectrum Chemical MFG), NMP (JT Baker), PEG (MW400 from JT Baker), PG (MP Biochemicals), TCL (Acros Organics), Brij30 (Sigma Aldrich), Brij98 (Acros Organics), UH, TU(Acros Organics), IPM (Alfa Aesar), Span20 (Sigma Aldrich.), sodium hydroxide(Sigma Aldrich), gentamicin sulfate(Sigma Aldrich), sodium phosphate dibasic(Acros Organics), formic acid (Acros Organics), triethylamine (Sigma-Aldrich),Franz diffusion cells and neoprene nail adapters (PermeGear), Cadaver skin and cadaver toenails (Allosource, Cincinnati and Anatomy Gifts Registry, Maryland), Nanopure water was used for preparation of 10mM ph7.4 phosphate buffered saline, HPLC grade acetonitrile (Fischer scientific). All the chemicals were used as received without any further purification.

2.4 Experimental Methods

2.4.1 Sample Preparation for analysis

The assay of EN in the nail was performed by first dissolving the nails in 0.3 mL of 1M sodium hydroxide solution for 24h in the water bath 32°C, then these solution were diluted sufficiently using acetonitrile to reduce the strength of 1M NaOH to 0.1 M, and filtered through Restek® 0.22μ syringe filters (cellulose acetate membrane) and analyzed according to the HPLC method.

2.4.2 High Pressure Liquid Chromatography

EN content in the samples was assayed using an Agilent 1100 series HPLC with variable wavelength detector. Acetonitrile: water (50:50 v/v) was used as the mobile phase with 0.5% formic acid and 0.1% triethylamine. The flow rate was 1 mL/min and the injection volume was 80 μL. The wavelength of detection for EN was 230 nm.

2.4.3. Recovery of Econazole nitrate from human nails

Solutions of EN in acetonitrile with concentrations 0.0625 mg/mL, 0.125 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 1.0 mg/mL and 2.0 mg/mL were prepared. Approximately 10 mg of human nail clippings were added to each concentration of EN solution. Each concentration was studied in triplicate. The nail clippings were treated with 200 µL of each of these solutions for 24 hr 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 with acetonitrile and the sample preparation method was followed to quantify the concentration of EN.

2.4.4. The Recovery of Econazole nitrate from epidermis and dermis

A stock solution containing 2 mg/mL of EN in acetonitrile was prepared. Solutions with the concentrations 0.0625 mg/mL, 0.125 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 1.0 mg/mL and were prepared using the stock solution. 50 µL of each of these solutions, including the stock contains 3.125µg, 6.25µg, 12.5µg, 25µg, 50 µg and 100 µg of EN respectively. Each of the above concentrations was studied in triplicate. 9 mm diameter skin sections 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 (Frank, J.D. et al 1995 and Kassis,V. and SØndergaard, J.1982). Both the epidermis and the dermis, for each skin section were separately treated with 50 µL of the drug solutions for 24 hr in microcentrifuge tubes. After the drug treatment, 1 mL acetonitrile was added to each of the microcentrifuge tubes and equilibrated for 24 hr for drug extraction at 37 °C. The extracting medium was filtered through 0.22 µ syringe filters and analyzed using HPLC.

2.4.5 Determination of the saturation solubility of EN in PEs

An excess amount of EN was added to 0.5 mL of each PE. The drug was equilibrated with the enhancers for a period of 72 hours at 32 ± 1 ºC. A saturated solution of EN in pH 7.4 PBS (10mM) served as the control solution. At the end of 72 hr, the solutions were filtered through a 0.22 μ syringe filter. The samples were analyzed using the HPLC method mentioned previously. The enhancement in solubility, EFsol, of Econazole nitrate in presence of the enhancers was calculated using the equation below

$$EF_{\mathrm{SOL}} = \frac{EF_{\mathrm{PE}}}{EF_{\mathrm{pH7.4PBS}}} \quad \text{..........(2.1)}$$

Where $[EN]_{PE}$ is equilibrium concentration of EN in presence of PEs and $[EN]_{pH\ 7.4\ PBS}$ is the equilibrium concentration of EN in pH 7.4 PBS (Control).

2.4.6 The in vitro nail Partitioning of EN

The PEs were screened for their ability to increase the concentration of the drug within human nail clippings. Human nail clippings were obtained from healthy volunteers (20-50 years of age) in the Department of Pharmaceutical Sciences, Temple University. The nail clippings were washed thrice with ethanol:water (70:30 v/v) and then rinsed with nanopure® water. The nail clippings were air dried at room temperature and stored at 2-8 ºC until use.The saturated solutions of EN in the PEs and 10 mM pH7.4 PBS were prepared. 14mg of previously washed and dried, human nail clippings were weighed into micro centrifuge tubes. The nail clippings were treated for 24 hours with 0.5 mL of each enhancer saturated drug solution at 32 ± 1 ºC. As a control, nail clippings were treated with a saturated solution of EN in pH 7.4 PBS (10mM). After 24 hour of drug treatment, the nail clippings were washed three times with 5 mL of nanopure water to remove any surface adherent drug. The nail clippings were dried using tissue paper and were dissolved in 0.3 mL of 1 M sodium hydroxide solution at 37 °C. The nail solutions were diluted using acetonitrile to a base concentration of 0.1M and filtered using 0.22 μ syringe filters. These solution were analyzed using HPLC as described in the previous sections. The enhancement factor, EFsim, was calculated to quantify the improvement in EN penetration into nail clippings relative to that in the control.

$$\mathrm{EFsim} = \frac{\mathrm{EN}_{\mathrm{simPE}(\mu g/\mathrm{mg\ of\ nail})}}{\mathrm{EN}_{\mathrm{sim\ pH7.4\ PBS}(\mu g/\mathrm{mg\ of\ nail})}} \quad (2.2)$$

Where $[EN]_{simPE}$ and $[EN]_{simpH\ 7.4\ PBS}$ are the concentrations of EN within the nail clippings in presence of PEs and pH 7.4 PBS (control) respectively.

2.4.7.The in vitro transungual permeation of econazole nitrate

The nail penetration study screens the different PEs for partitioning into the nail. However, the improvement of nail permeation must be confirmed using an in vitro transungual permeation study. The in vitro transungual permeation of EN was performed using only the PEs which produced a significant increase in nail partitioning. The human cadaver toenails (Anatomy Gifts Registry) were washed with nanopure water and blotted with tissue paper. The weights and thicknesses of the toenails were measured. The human cadaver toenails were hydrated at 100 % RH for 24 hr, prior to mounting on Franz diffusion cell with nail adapters. Then cut it into size 5mm*5mm square to just cover the orifice, this size is to prevent the lateral diffusion of the drug. The nail adapters (3 mm orifice diameter) with toenails were placed between the donor and receiver compartments of the Franz cells. The receiver compartment was filled with 3 mL of pH 7.4 PBS containing 0.1 % w/v gentamicin sulfate. Gentamicin sulfate was added to prevent microbial growth in the receiver compartment. The donor compartment was dosed daily with 20µL of saturated solution of EN in the potential enhancer from the nail penetration experiment. The temperature of the receiver compartment solution was maintained at 32 ± 1 °C for the duration of 28 days. The study was performed under occlusion by covering the donor compartment with parafilm. On days 1, 2, 4, 8, 12, 16, 20, 24 and 28, 0.3 mL of the receiver compartment solution was sampled and analyzed for drug content.

Additionally, on day 28, the nails were dismounted from the nail adapters and the drug content was quantified by separating the nail just below the orifice and the nail surrounding the orifice. The drug quantification was performed using the process described in the sample preparation.

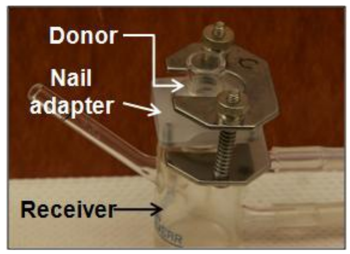


Figure 2.2: Franz diffusion cell with the nail adapter between the donor and receiver compartments

Figure 2.3: Neoprene nail adapter with 3 mm orifice diameter

2.4.8. The in vitro skin permeation of econazole nitrate

The in vitro skin permeation of EN was performed using the PermeGear Franz diffusion apparatus with the internal volume of 3 mL and orifice diameter of 9 mm. Dermatomed human cadaver skin (Allosource) was thawed at room temperature for 15 minutes. Skin pieces (2.25 cm^2) were cut and placed in pH 7.4 PBS for 20 min to remove the glycerin used for cryopreservation of the cadaver skin. The skin pieces were blotted with tissue paper and placed between the donor and the receiver compartments. The receiver compartment was filled with 3 mL of pH 7.4 PBS and the skin was allowed to equilibrate with the receiving medium for 60 min, prior to drug dosing. 10µL of the saturated solution of the drug in the PEs was added to the donor compartment to form a thin film on the surface of the skin. The receiver compartment solution was maintained at 32 ± 1 ºC, for a period of 24 hours. Each cellswas occluded by covering the donor compartment with parafilm. Each of the PEs was tested in triplicate and the data was compared thethe control. The control cells were dosed with 10 µL of saturated solution of EN in pH 7.4 PBS. At 2, 4, 6, 8, 22 and 24hr, 0.3 mL of the receiver compartment solution was withdrawn and replaced with 0.3 mL of fresh buffer to maintain sink conditions.

The drug content in the samples was analyzed using the HPLC method. The steady state flux (J) for permeation (µg/cm²/hr) was obtained from the slope of the linear portion of the plot of cumulative amount of EN permeating per unit surface area of skin (µg/cm²) against time (hr). The permeability coefficient Kp was calculated from the ratio of the steady state flux (J) and the concentration in the donor compartment (Cd). Since saturated solutions were used as donor solutions, the saturation solubility for Cd of each EN. The enhancement in flux for skin permeation (EFflux) was calculated using the equation:

$$\mathrm{EF_{flux}} = \frac{\mathrm{Flux_{PE}\ of\ EN\ permeation\ (\mu g/cm^2/hr)}}{\mathrm{Flux_{pH\ 7.4\ PBS}\ of\ EN\ permeation\ (\mu g/cm^2/hr)}} \quad \text{...(2.3)}$$

Where $\mathrm{Flux_{PE}}$ and $\mathrm{Flux_{pH\ 7.4\ PBS}}$ are the stead state flux values for permeation of EN across the skin in presence of the PEs and pH 7.4 PBS (control) respectively.

2.4.9. Finite dose skin penetration of econazole nitrate

The skin used in the finite dose permeation study was further analyzed for drug content by separating the epidermis and the dermis. Post the finite dose skin permeation experiment, the 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; the area exposed to the drug was separated from the peripheral The epidermis was then peeled from the dermis using sharp forceps. The two layers were then placed in separate glass vials containing 0.3 mL of acetonitrile each at 37 ºC for 24 hr to extract EN. 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 enhancement factors, EFepidermis and EFdermis give the increase in accumulation of EN in the epidermis and dermis relative to the control.

$$\frac{\mathrm{EFepidermis}}{\mathrm{EFdermis}} = \frac{[\mathrm{EN}]\mathrm{PE}\ (\mu\mathrm{g/mL})\ \mathrm{in\ epidermis\ ordermis}}{[\mathrm{EN}]\ \mathrm{pH\ 7.4\ PBS}\ (\mu\mathrm{g/mL})\ \mathrm{in\ epidermis\ or\ dermis}} \quad \text{....................................(2.4)}$$

Where [EN]PE and [EN]pH7.4 PBS are concentrations of EN within the epidermis/dermis in presence of PEs and pH 7.4 PBS (control) respectively.

2.4.10. Drug – keratin binding

Drug-keratin binding was performed using keratin powder. Prepare for serial dilution solution of the selected PE in drug. Approximately 10 mg of keratin powder was used for each sample. Each of the concentrations was studied in triplicate. The weighed keratin powder (spectrum chemicals) was added to 2 mL micro centrifuge tubes. To each tube 0.3 mL of the drug solutions were added and the tubes were placed in the water bath at 32 ± 1 ºC for 48 hr. Each of the samples was emptied into micro-centrifuge tubes containing 0.22 µm filter cartridges (Ambion). The samples were centrifuged at 10000 rpm for 10 min. The unbound concentration of EN in the filtrate was quantified using HPLC.

The bound concentration of EN was calculated by subtracting the unbound concentration from the incubation concentration. The incubation concentration and the bound concentration of EN were normalized with the weight of keratin powder in the sample and plotted.

2.3.11 The pH effect on EN Solubility

Because EN is a weak base, the solubility of EN will decrease with the pH increase. Buffer with pH3.6 (10mM), pH5 (10mM) and pH7.4 (10mM) were prepared. These were added to each PE with an increased solubility Ef. Generally the PE concentration tested was 10%. Pure buffer was used as a control. We will do the same process as above to see how different pH will affect the solubility of EN, the nail penetration and also the skin penetration of EN and also the keratin binding.

2.3.12. Statistical Analysis of Experimental Data

All the experiments were performed in triplicate and the data was reported as mean value (± standard deviation). A One-Way ANOVA with Tukey's Test ($p=0.05$) was used to determine significant differences. (JMP Pro version 10.0)

2.5 Results and Discussion

2.5.1. High Pressure Liquid Chromatography

Linear plot in the range of 0.09 to 100 ug/mL with a correlation coefficient of 0.9987. The retention time is 3.8 ± 0.08 min. The CV, limit of quantification (LOQ) and limit of detection (LOD) for EN are 0.153, 90 ng/mL and 29 ng/mL respectively.

2.5.2. Recovery of Econazole nitrate from human nail

A recovery of 96.5-99.4 % was obtained for EN in the concentration range of 1.0 µg/mg nail to 40.0 µg/mg nail after dissolution in 1 M NaOH and extract from acetonitrile.

2.5.3. Recovery of Econazole nitrate from epidermis and dermis

87.3 to 96.2 % of Econazole nitrate was recovered when epidermis with a diameter of 9 mm was treated with 3.125 µg to 100 µg of EN after the extraction of the epidermis. The recovery for the dermis was greater than 95 %.

2.5.4. Saturation solubility of Econazole nitrate in PEs

Figure 2.4 shows that the solubility of EN was significantly higher TCL, PEG, PG, also Brij30, Brij98, UH, NMP and TU is also higher compared to the control (pH 7.4 PBS). The nail PEs IPM and Span20 did not increase the solubility of EN. An increase in solubility of EN in penetration enhancer increases concentration gradient across the skin. EN solubility in the enhancers was determined to elucidate any correlation between improvement in permeation and/ or penetration into the target tissues.

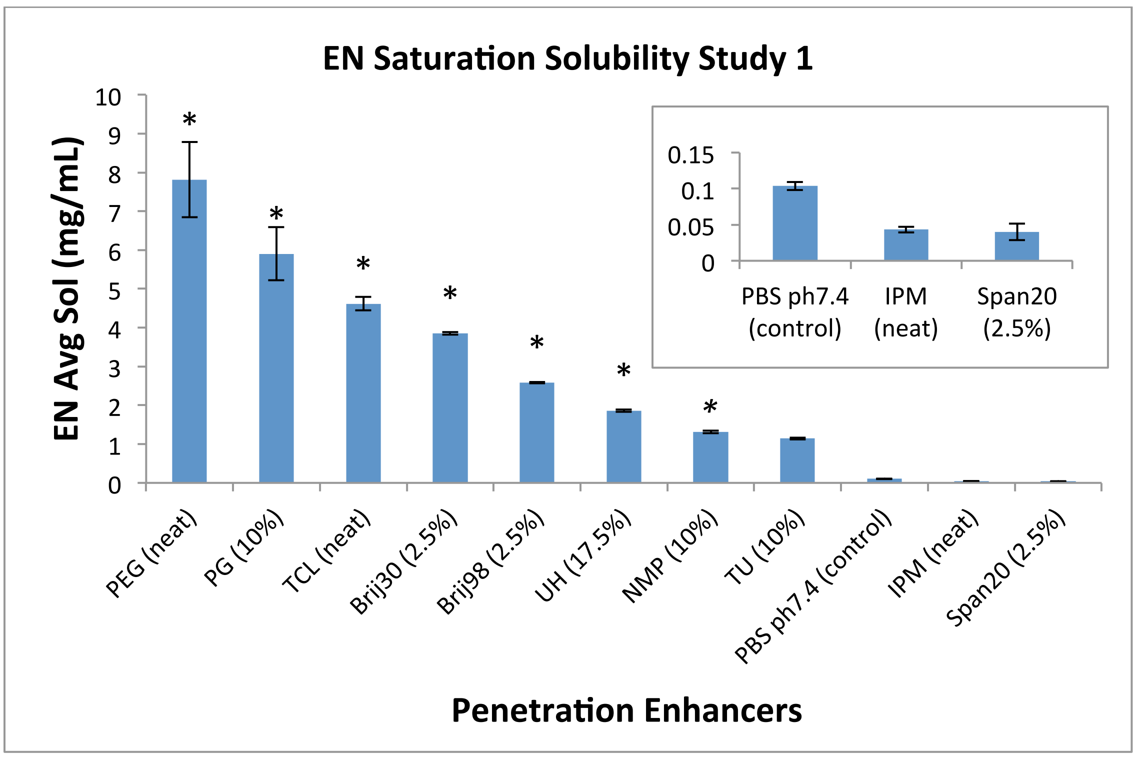


Figure 2.4: Saturation solubility (mg/mL) of EN in presence of PEs

2.5.5 In vitro nail partitioning of Econazole Nitrate

This was a simultaneous study, which means expose the nail clippings to the PEs and the drug solution at the same time. From the graph we can see TU, NMP significantly increase the drug accumulation in nail clippings. Brij98, Brij30 and PG, UG also increase the drug accumulation in nail clippings but to a lesser extent. TCL, PEG and Span20 are decrease nail partitioning. PEs act by various mechanisms, one of the methods of action of the PEs is increasing the drug solubility. This increases the concentration gradient of drug across the nail plate and the skin. From the study we can tell the increasing of solubility is just one mechanism. A more important mechanism maybe the increase in partitioning into the nail and skin. For example, TU can reduce disulphide linkages in keratin of nail, which would be a superiority choice because the keratin is a key structural component in human nails.

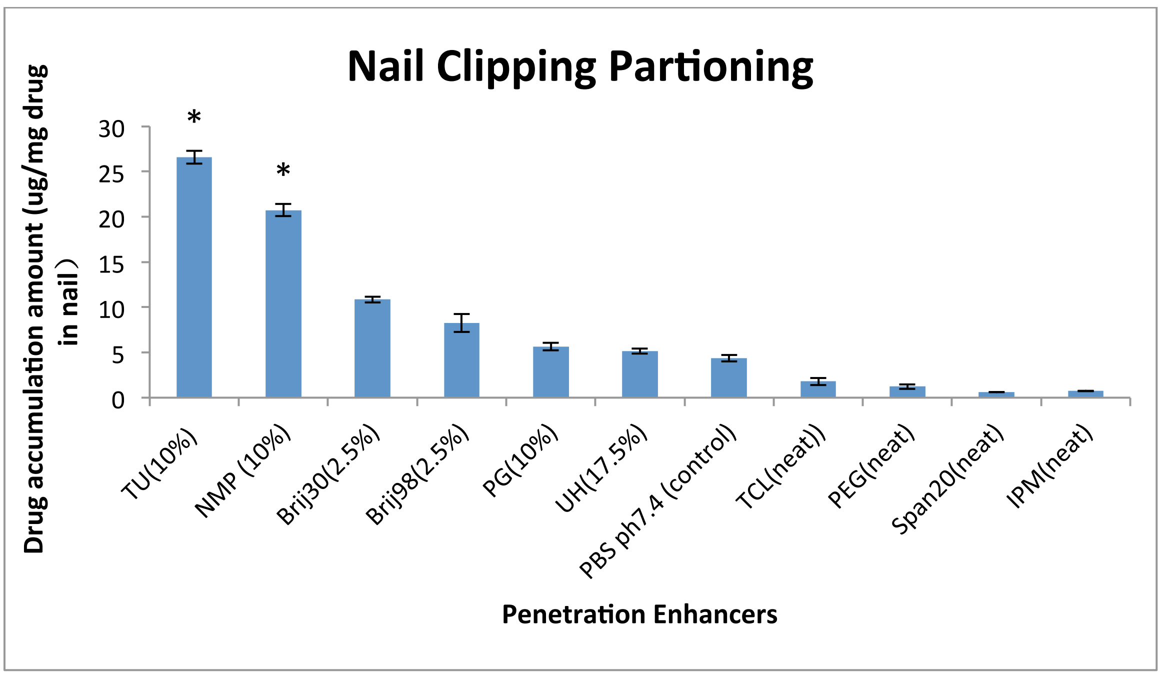


Figure 2.5: Concentration (µg EN/mg nail) of EN in the nail clippings in presence of PEs (simultaneous exposure study)

After we study both how PEs will affect the solubility of EN and EN accumulation in nails, an analysis of the relationship between EFsol and EFsim was conducted. EFsol wads ploted verses EF sim. By definition, the solubility and partitioning of PBS ph7.4 is the (1,1) control coordinate. The resultant graph has four quadrants. Of interest are PE which are located in Quadrant III. These PE increase solubility and increase partitioning of the drug into the nail. TU, NMP, Brij98, Brij30 and PG, UH were the favored PE in Quadrant III. From this graph, it is observed that the ability of PEs to increase solubility is not related to the ability to increase the drug penetration into the skin. EFsol and EFsim are separate factors which will act by different mechanisms.

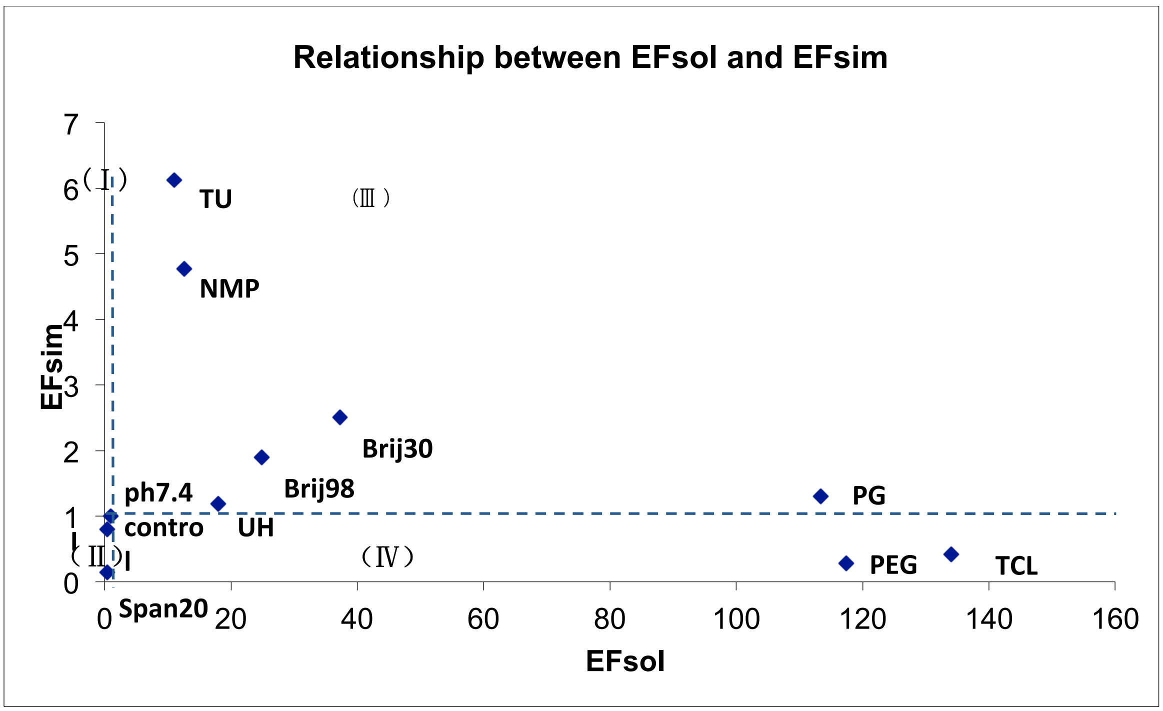


Figure 2.6 A: Analysis plot showing the relationship between the enhancements in solubility and nail penetration of EN in presence of PEs.

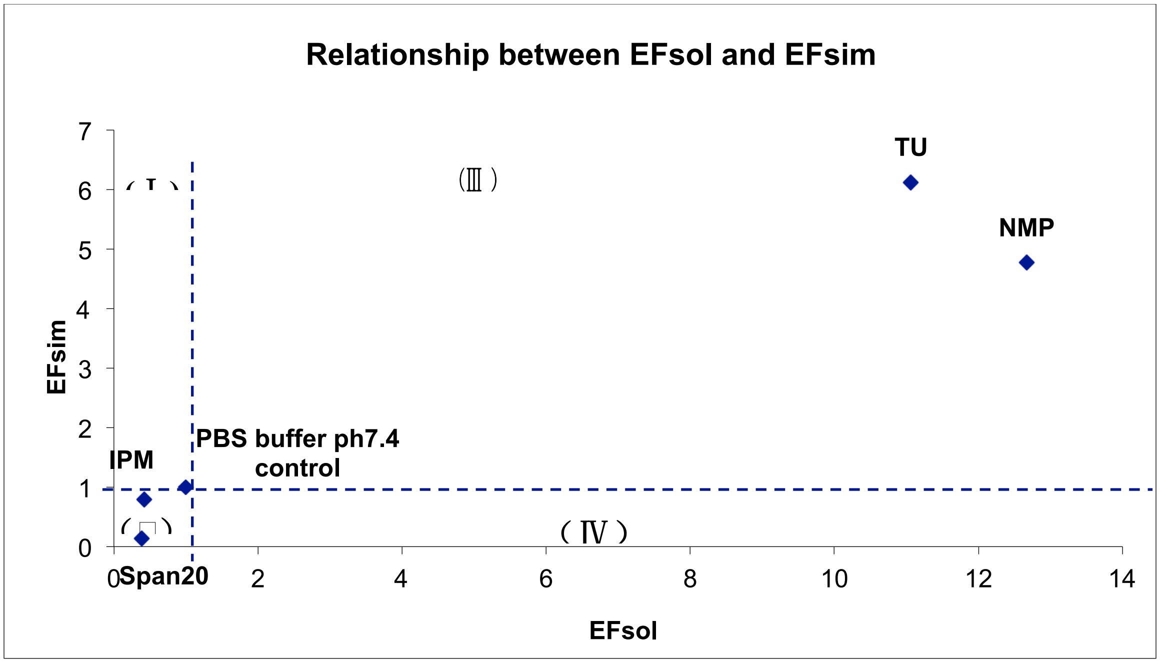


Figure 2.6 B: Analysis plot showing the relationship between the enhancements in solubility and nail penetration of EN in presence of PEs.

After observation of the raw data between EN saturated solubility and drug accumulated amount in nail clippings, it's observed that there's an optimal range, the PEs with the solubility around 1mg/ml (eg. TU, NMP) have the highest drug partitioning into nail clippings. In the drug permeation process, it is desirable to have a high solubility of the drug to ensure there enough driving force to push the drug into the nail. But this high solubility may cause the drug to stay in the solution rather than partition into the nail. So a balanced, optimal PE is desired. This PE which can enhance the solubility of EN without decreasing partitioning. To model this optimal solubility a log solubility verses drug accumulation plot was constructed. It was hypothesized that this would produce a bell-shaped curve (figure 2.7). , The PEs which can enhance the EN to have the solubility around 1mg/ml can have the best ability to penetration into the nails, others are either in the left or right side downside of the curve.

This observation can help the further experiment to predict the ability of PEs to penetrate into nails according to their ability to enhance the solubility of EN. The PEs which have an EN solubility around 1mg/mL will have the strongest ability to penetrate the drug into nails.

Table 2.1: Raw data of EN saturated solubility, drug penetration amount and Log scale solubility

Sample	Avg Sol (mg/ml)	Accumulation in nail clippings (ug/mg)	Log solubility
PEG (neat)	7.814415064	1.228321456	0.892896
PG (10%)	5.901259569	5.650764993	0.770945
TCL (neat)	4.612004071	1.796114493	0.66389
Brij30 (2.5%)	3.851104516	10.86338305	0.585585
Brij98 (2.5%)	2.579840289	8.24683892	0.411593
UH (17.5%)	1.864494371	5.14836051	0.270561
NMP (10%)	1.310668555	20.72817631	0.117493
TU (10%)	1.14464828	26.60362306	0.058672
PBS ph7.4 (control)	0.103448453	4.344744019	-0.98528
Span20 (2.5%)	0.040056638	0.622213964	-1.39733
IPM	0.04315042	0.7356788	-1.36501

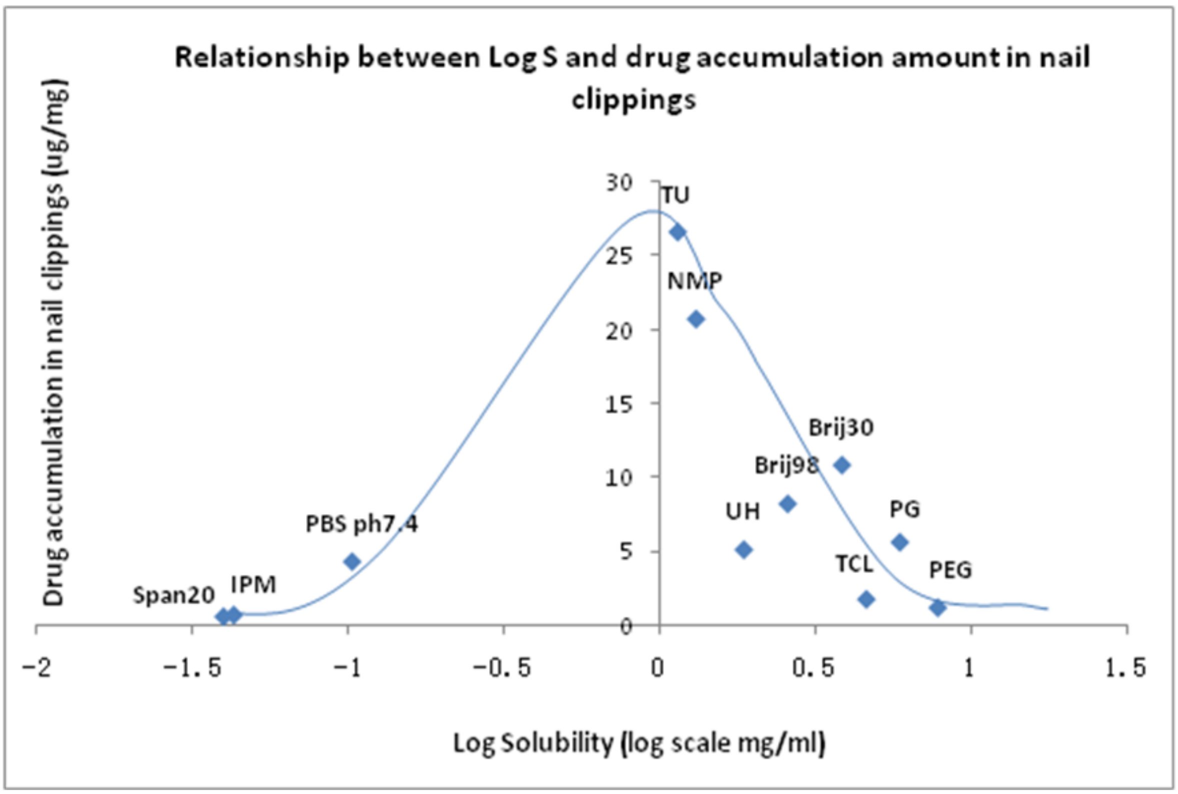


Figure 2.7: The relationship between Log scale solubility and drug accumulation amount in nail clippings

2.5.6. In vitro skin penetration

The skin penetration study quantifies the levels of the drug in the epidermal and dermal layers of the skin. One of the target sites for the topical antifungal therapy is the skin, specifically the epidermis. Here the superior choice will be a PE, which will have higher accumulation drug amount in the epidermis and lower accumulation in the dermis. This will ensure sufficient amount of drug at the target tissue and less drug in the systemic circulation.

The data in figure 2.8 is arranged in the decreasing order of the enhancement factor in the epidermis. Any value greater than 1 for enhancement factor is desirable in the epidermis because the superficial cutaneous infections resides in the epidermis. Figure 2.7 shows the enhancement in accumulation of EN in epidermis and dermis of the skin in the presence of the enhancers. TU, NMP, Brij30 and PG showed 1.44, 1.39, 1.21 and 1.04 fold enhancement in drug levels in the epidermis. The same agents showed 0.7, 2.2, 2.7 and 1.17 fold improvement in drug levels within the dermis. TU was the only agent showed higher accumulation of EN specifically in the epidermis and a low accumulation in the dermis. The blood capillaries are located in the lower layers of the dermis. If the level of the drug increases in the dermis, it is probable that this drug will eventually partition into the blood. Considering the epidermis and dermal concentrations, TU would be the best choice for skin.

Figure 2.9 also shows the relationship between EFepidermis and EFsol. TU is in the third quadrant, showing a 11-fold improvement in solubility and 1.44 fold increase in EN levels in the epidermis. Despite of higher drug accumulation in the epidermis and dermis in presence of NMP and B30, these PEs are not preferred for further formulation development, because they also increase the accumulation of EN in the dermis. This could lead to other toxicities. Therefore, it is important to choose PEs which show greater accumulation in the layers of the skin with minimum flux for the drug permeation. TU was chosen as the best enhancer for the skin penetration of EN, because it showed much lower dermis drug accumulation than the control and enhanced the levels of EN, selectively within the epidermis.

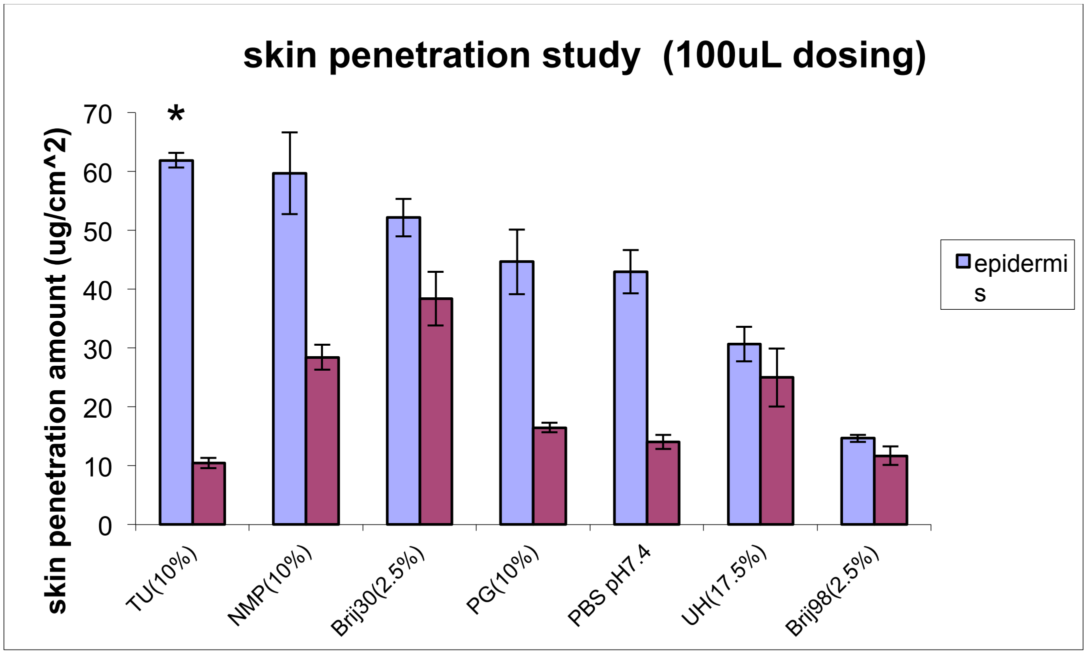


Figure 2.8: Accumulation of EN within epidermis and dermis in presence of PEs

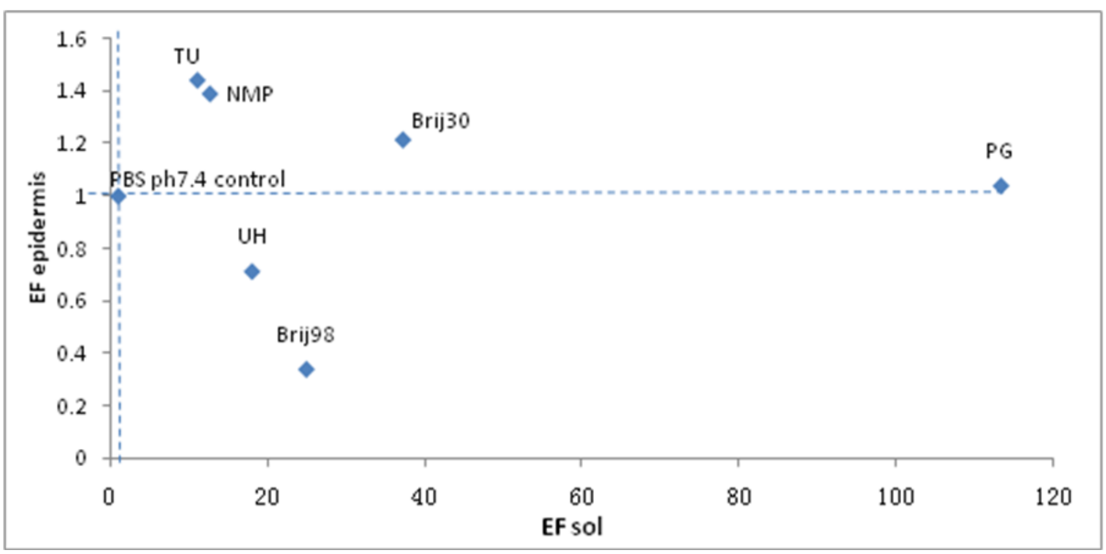


Figure 2.9: Analysis plot showing the relationship between the enhancement factors in solubility and epidermal accumulation of EN in presence of PEs

2.5.7 In vitro skin permeation

After 20uL dose to the skin for three different treatments (pH7.4 PBS buffer, pH5 PBS buffer, TU 10% in pH5 buffer), no drug amount appeared in the receiver compartment after 24 hours. This can be explained by the high LogP of EN, the drug EN would prefer to stay in the Stratum Corneum, which is a hydrophobic instead of diffusing into the buffer. This will be beneficial because the main goal of topical delivery is to maximize skin exposure and minimize the systemic exposure of the drug. This reduces the chances of adverse side effects and drug-drug interactions.

2.5.8 The pH effects on PEs

The previous results show that TU would be the best choice for EN, because it has higher EN accumulation in nails, epidermis, and lower drug accumulation in the dermis. A NMP would be the good choice however it does not as good an EF as TU. The effect of pH on the solubility of EN in TU and NMP was performed. EN is a weak base drug, so with an increase will lead to more unionized drug which will lead to lower aqueous solubility. From the graph below we can see with the only buffer, TU10% in different pH buffer and NMP10% in different pH buffer, the three groups all have the same trend, which is with the pH increase, the EN solubility decreases. Higher solubility will ensure higher gradient between donor side and target tissue, it will push the drug into the target tissue along the concentration gradient; this could be an advantage for the formulation.

Initially, the solubility studies were performed in citrate and acetate buffers. The solubility of the EN was lower at pH 5 than pH 7.4 for those buffers. (Figures-2.11, 2.12, 2.13, 2.14) This observation was confirmed by repeating the study.

It was hypothesized that anionic organic acids may actually decrease the solubility of EN at pH 5.0. This can be as simple as the anionic buffer combining with the cationic drug to form another salt.

To correct this a phosphate: ammonium hydroxide buffer, which has an optimal buffer capacity at pH 4.8, was used. This produced the hypothesized results which have EN with aqueous solubility in the rank order pH 3.5> pH 5.0 > pH 7.0. (Figure 2.15)

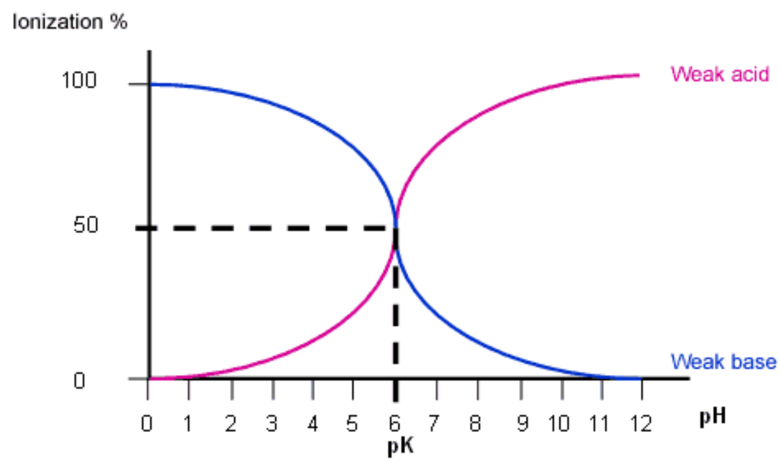


Figure 2.10: The relationship between pKa and ionization status for weak acid and weak base

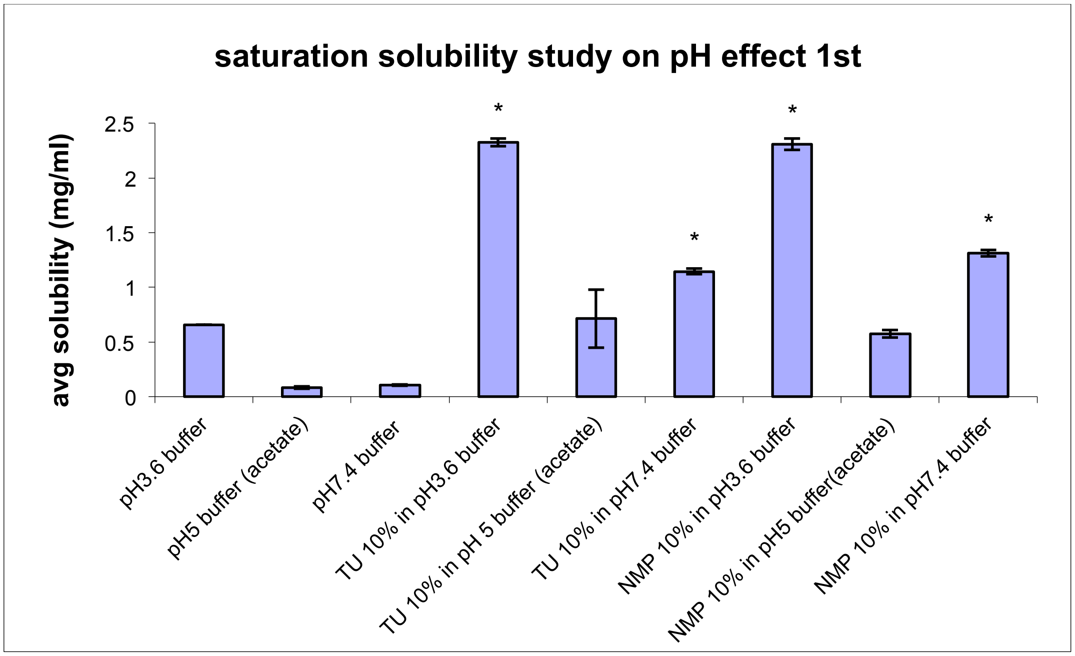


Figure 2.11: Saturation solubility (mg/mL) of EN in presence of selected PEs in different pH buffer 1st trial

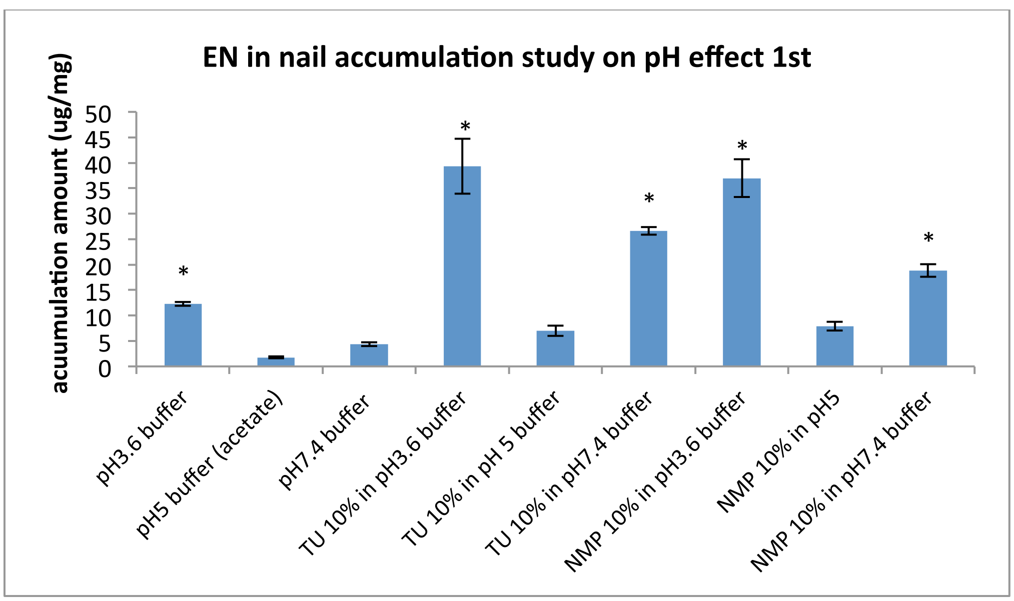


Figure 2.12: Concentration (μg EN/mg nail) of EN in the nail clippings in presence of selected PEs in different ph buffer 1st trial

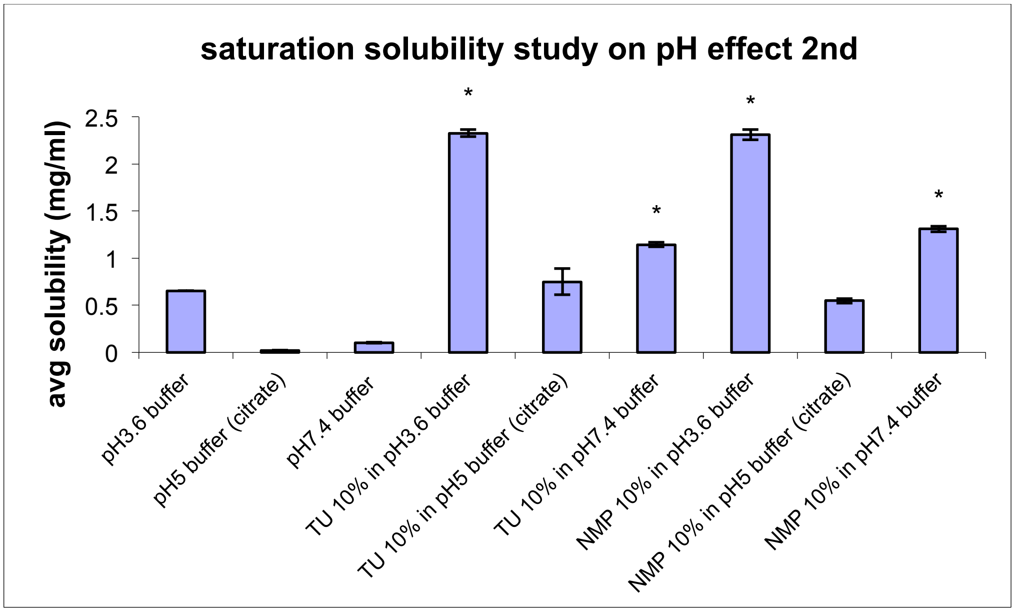


Figure 2.13: Saturation solubility (mg/mL) of EN in presence of seleted PEs in different pH buffer 2nd trial

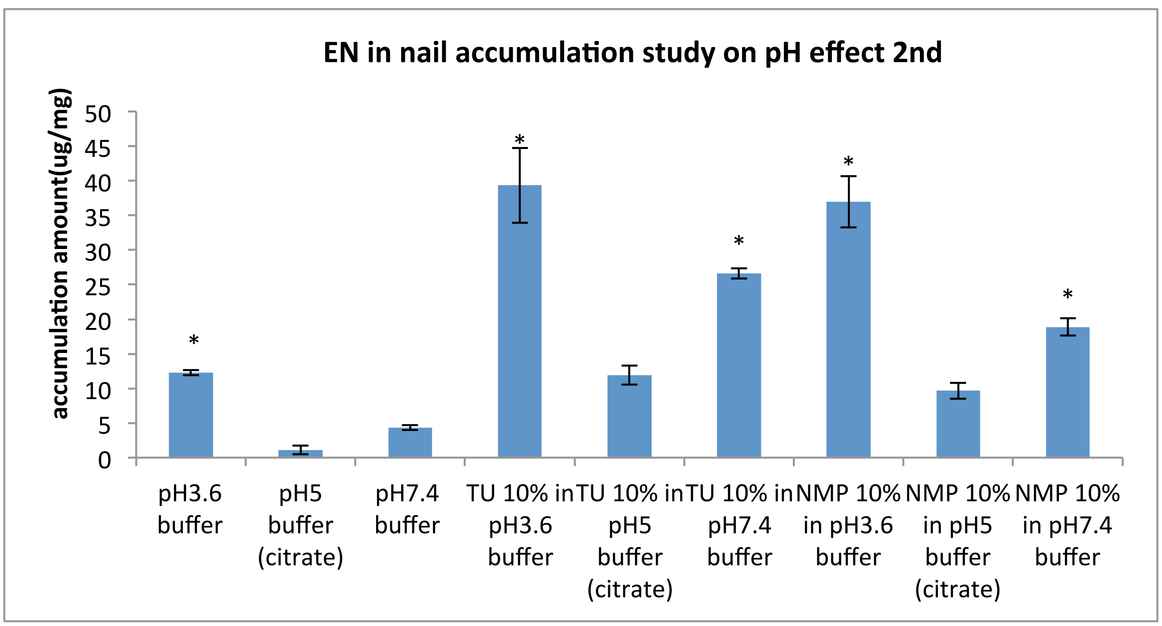


Figure 2.14: Concentration (µg EN/mg nail) of EN in the nail clippings in presence of selected PEs in different ph buffer 2nd trial

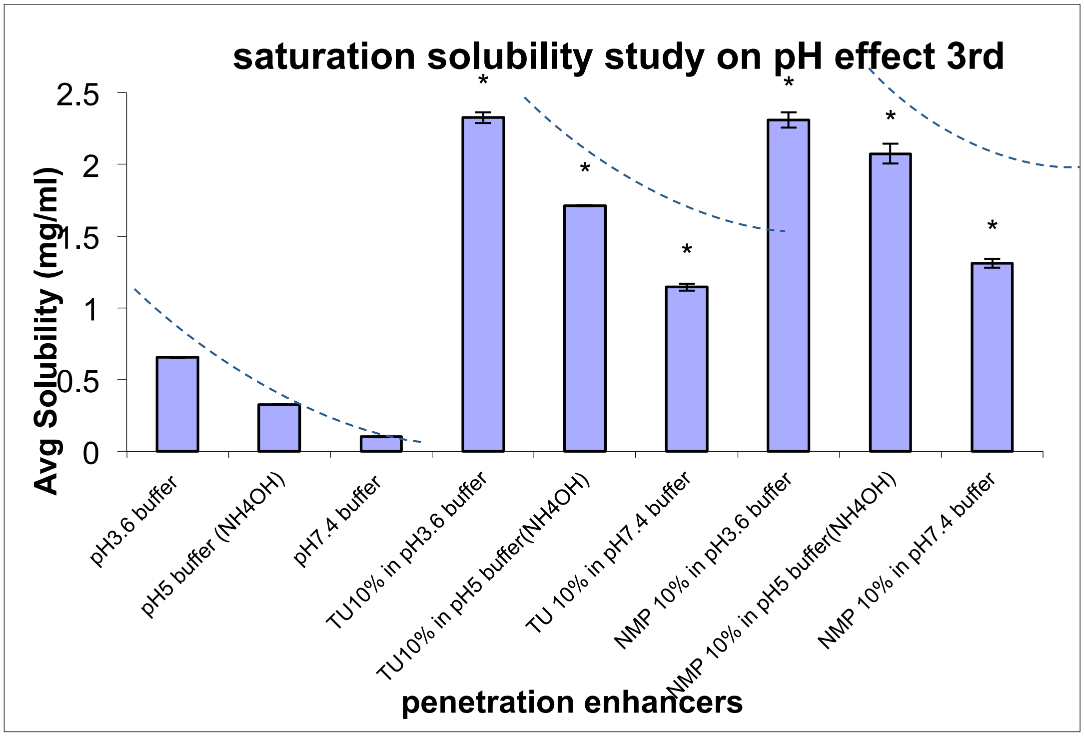


Figure 2.15: Saturation solubility (mg/mL) of EN in presence of seleted PEs in different ph buffer 3rd trial

Below is nail partitioning study for selected PEs (TU and NMP) in different pH's (Figure 2.16). The nail partitioning study has the same trend solubility. (pH 3.6> pH5.0 > pH 7.4) Additionally, the nail portioning improves with the use of the PE's TU and NMP. Again, the trend of TU producing increased effect over NMP is confirmed by these results.

Subsequent experiments focused on ph5 buffer and ph7.4 buffer. pH 3.6 buffer was eliminated from consideration because of the possibility of skin irritation at that pH. This would make it unfavorable for formulation consideration.

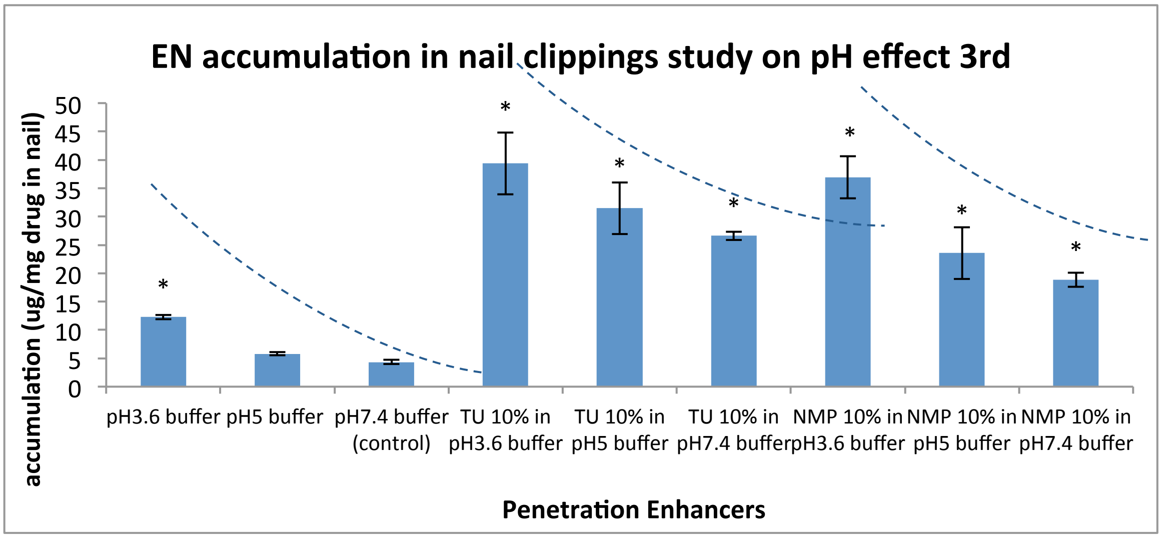


Figure 2.16: Accumulation (µg EN/mg nail) of EN in the nail clippings in presence of selected PEs in different pH buffer 3rd trial

The graph below is the skin penetration study with TU and NMP in pH5 and pH7.4 buffer. Three observations can be made from these results. First, the drug concentrations are greater using pH 5.0 compared to pH 7.4. Second, TU and NMP both increase the amount of drug in the skin over use of just thee buffer. Third, the trend of TU producing better enhancement compared to NMP is again confirmed.

Additionally, it can be seen that the pH did not produce significantly higher concentrations in the dermis. This means that there is no sacrifice of safety by decreasing the pH.

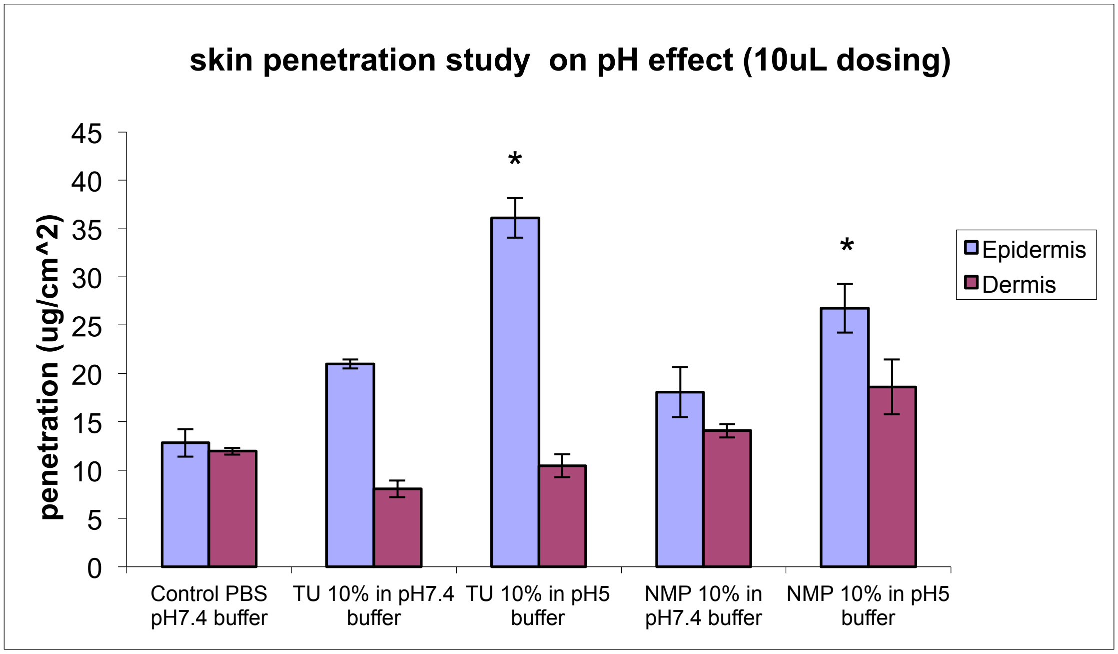


Figure 2.17: Penetration of EN within epidermis and dermis in presence of selected PEs in different pH buffer

2.5.9 The in vitro transungual permeation of econazole nitrate

The ability of TU in enhancing drug penetration was confirmed in the in vitro transungual permeation of EN through human cadaver toenail. The toenails, obtained from two male donors (79 and 81yrs of age) were smooth on the surface, normal and devoid of any symptoms (excessive thickness, dystrophy and discoloration) of onychomychosis. The average thickness of the toenails was 0.50 ± 0.07 mm. Figure below shows the permeation profile of econazole nitrate through human cadaver nail.

In this study PBS pH7.4 buffer was the control.pH5 buffer with and without 10% TU was evaluated for nail permeation. Each condition was performed in triplicate.

pH 7.4 and pH5 buffer have a lag time of around 2days. (Figures-2.18, 2.19) For TU10% in pH5 buffer, there's no observed lag time. The order of the permeation is 10% TU at pH 5.0 > pH 5.0 buffer=pH 7.0 buffer. This shows that produces a true enhancement of nail permeation.

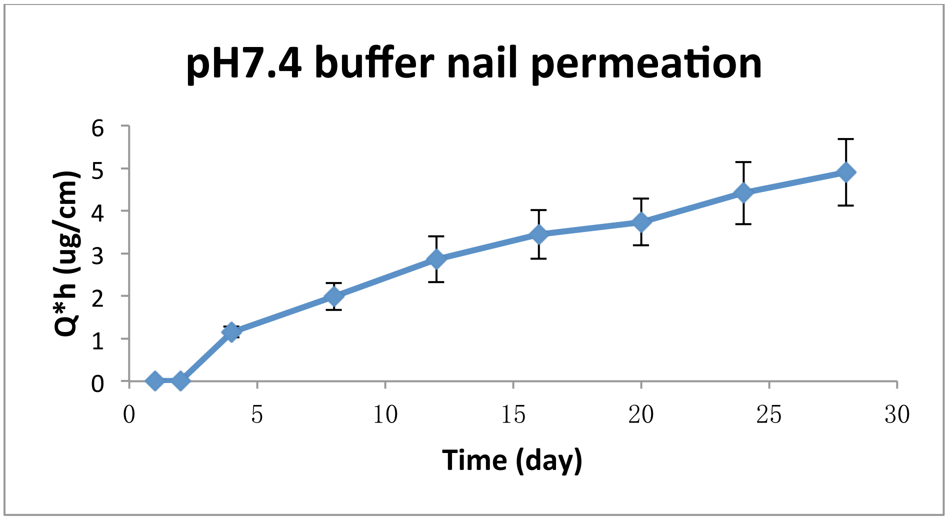


Figure 2.18: Transungual permeation of EN in presence of pH7.4 PBS buffer

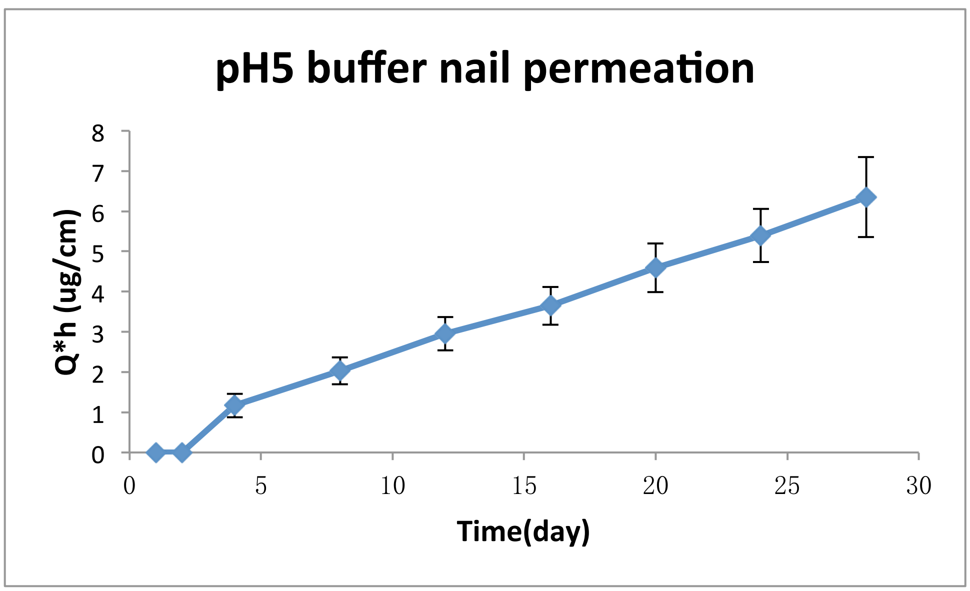


Figure 2.19: Transungual permeation of EN in presence of pH5 PBS buffer

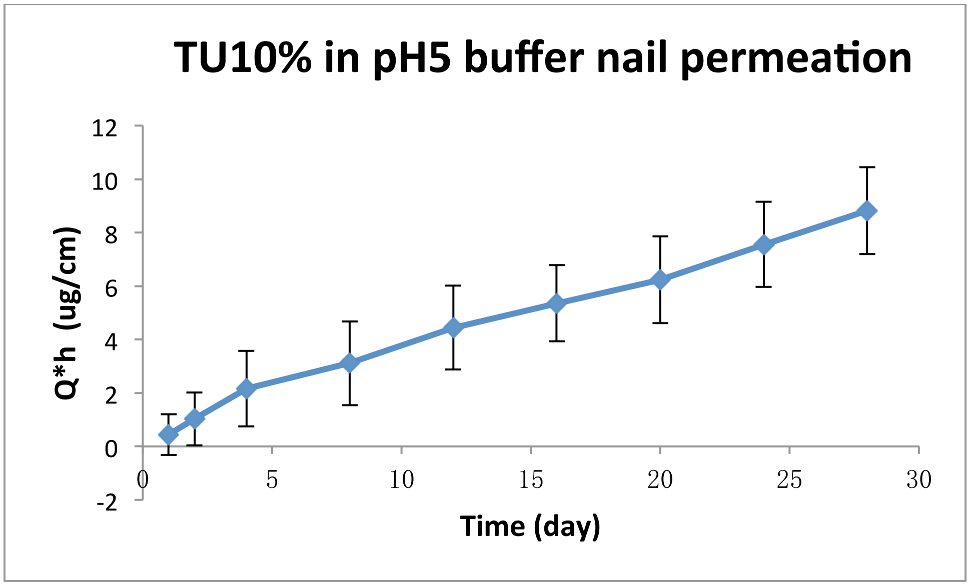


Figure 2.20: Transungual permeation of EN in presence of 10% TU in pH5 PBS buffer

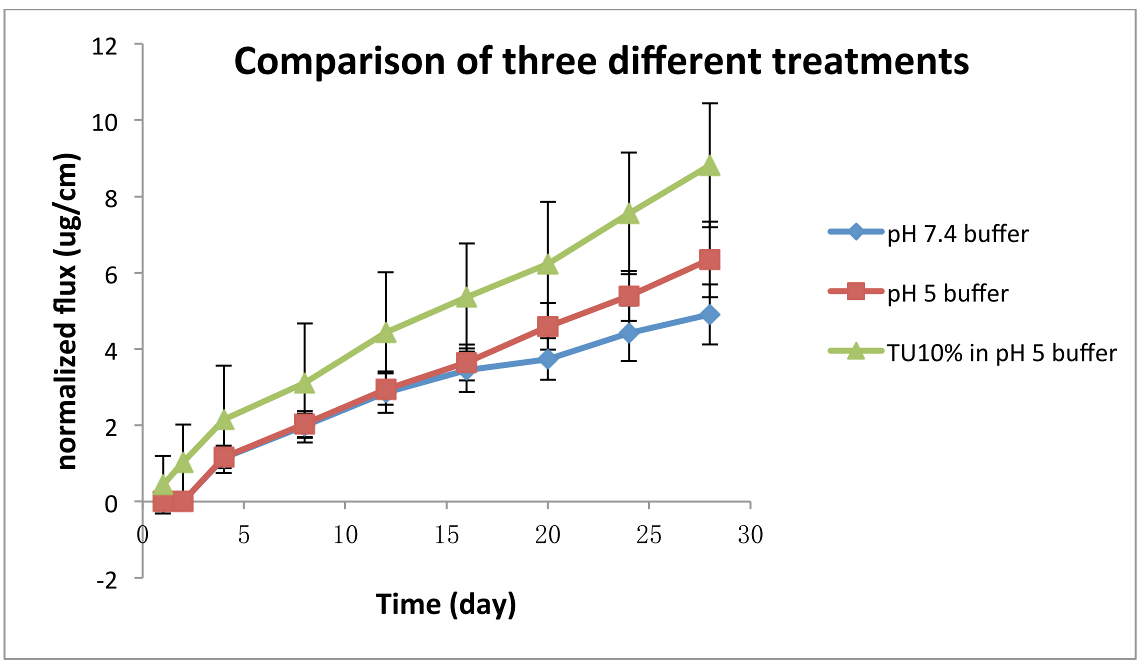


Figure 2.21: Combination of three treatments transungual permeation of EN

From the above figures the lag times were calculated from the slope of the terminal data. The lab time for pH 7.4 buffer was 0.843 days and for pH 5.0 buffer it was 0.176 days. No significant lag time was observed for 10% TU in pH 5.0 buffer. The pH 7.4 and pH 5.0 bufferes had nearly identical permeation profiles. The pH 5.0 buffer was significantly great at terminal data points. It is hypothesized that this is because the solubility increase is minimal. This would mean that the increase would only be observed in later time points where the differences in permeation become more magnified.

2.5.10 Drug-keratin binding

Figure below shows the binding of EN to keratin powder in the presence of 10% TU in different pH buffers. EN shows linear binding over the concentration range studied. In the presence of TU, a greater number of binding sites may become available for binding with EN. This is because the secondary and tertiary configurations of keratin are modified in the presence of disulfide bond breaking agents. The literature suggests that antifungal agents which show extensive keratin binding in the nail show reduced activity (Tatsumi, Y. et al 2002).

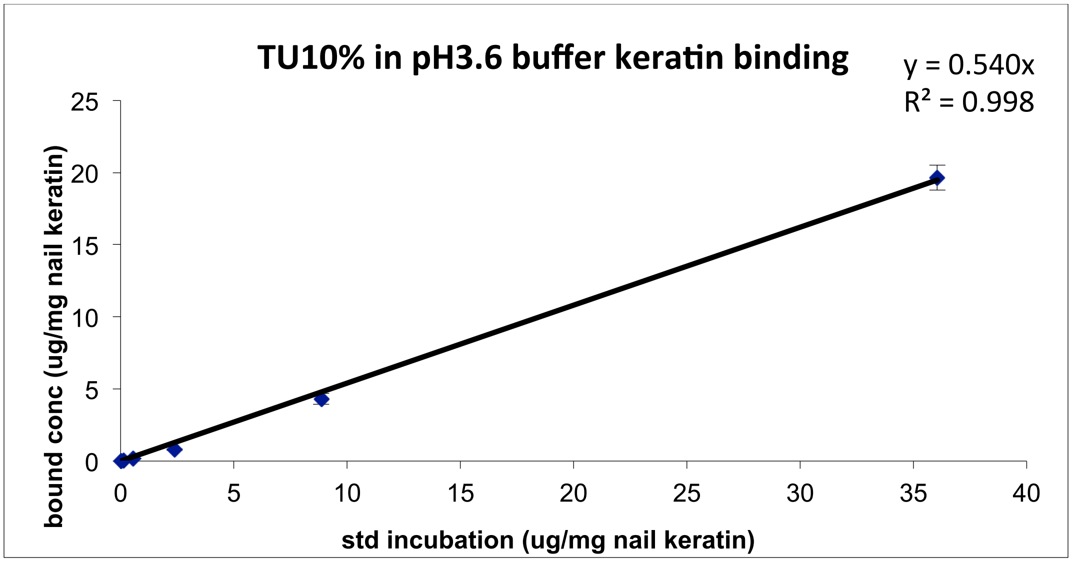


Figure 2.22: Concentration of keratin bound EN against the standard incubation concentration in presence of 10% TU in pH3.6 PBS buffer

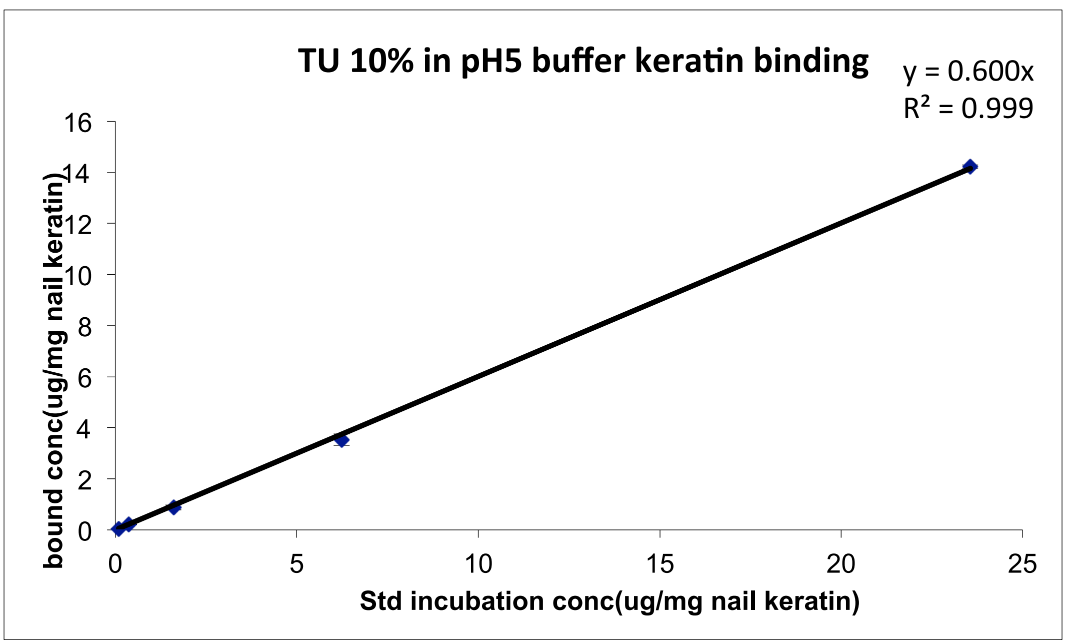


Figure 2.23: Concentration of keratin bound EN against the standard incubation concentration in presence of 10% TU in pH5 PBS buffer

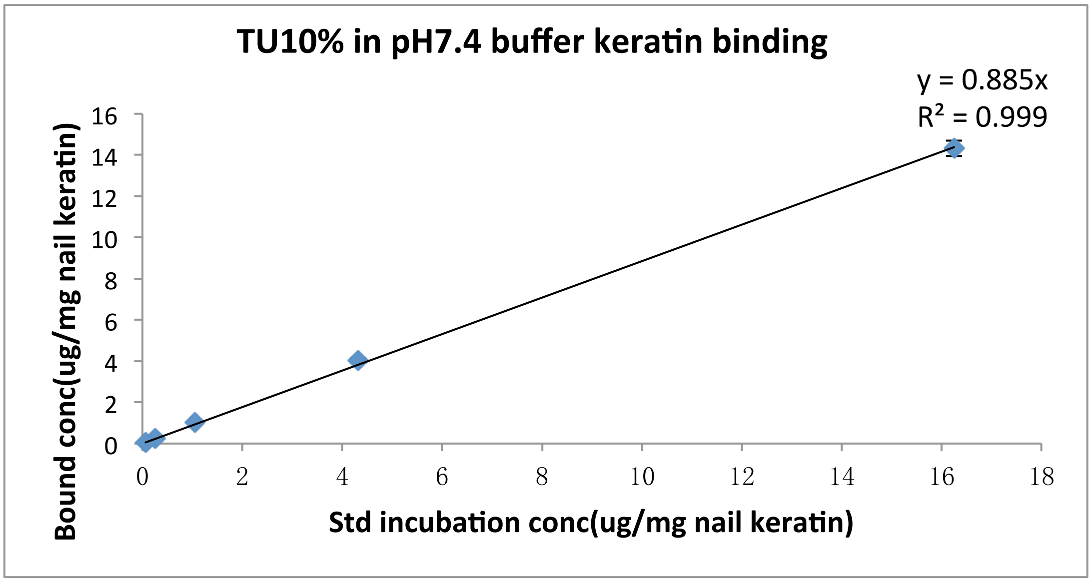


Figure 2.24: Concentration of keratin bound EN against the standard incubation concentration in presence of 10% TU in pH7.4 PBS buffer

Keratin binding reduces the amount of free drug for diffusion. Figures 2.22, 2.23, 2.24 above show that with a pH increase, the keratin binding increases. This can be explained by with the pH decreases, the uncharged form of EN decreases, and in EN's two forms (charged and uncharged form), uncharged form has more keratin binding. Thus, as pH is decreased the binding of EN to keratin also decreases. This would product more free drug for diffusion.

This shows that TU10% in pH5 buffer is better than TU10% in pH 7.4 buffer because it has lower keratin binding which is 60% compared to 88.5%. It will allow more free drug get into the target tissue and have more ability to kill the fungus. TU10% in pH3.6 buffer as already discussed because this low pH will cause irritation to skin.

CHAPTER 3

CONCLUSIONS

Topical treatment of onychomycosis is challenging because of the compact nature of the nail barrier and limited drug permeability across the nail. This limits the use of topical formulations for drugs that have a very low MIC and then they may only be useful to treat mild to moderate onychomycosis. There is an unmet need to develop an efficient topical formulation to extend its utility to treat moderate to severe stages of the disease. Econazole nitrate is an antifungal medication of the imidazole class, it inhibits of fungus synthesis results in increased cellular permeability causing leakage of cellular contents. it shows and favorable physicochemical properties of low molecular weight, balanced hydrophilic and hydrophobic property. It's an effective antifungal drug. In dermatological studies about 90% of a large number of patients were cured, often after a relatively short treatment period (2 to 6 weeks). (R.C.Heel, R.N.Brogden, 1978)

The pre-formulation strategy to improve topical delivery of Econazole nitrate into the human nail and surrounding skin (nail folds) is discussed.

In passive diffusion of the drug, the concentration gradient is the driving force for drug diffusion. It is therefore important to maintain a high concentration gradient between the formulation and the target tissues. This is achieved with the use of specific PEs, which also have other underlying mechanisms towards the target tissues. A pre-formulation strategy has been proposed for the selection of appropriate PEs for drug delivery into the nail plate and the nail folds. The saturation solubility experiments identified PEs which increase the solubility of econazole nitrate and can be potential enhancers. The aim of the study was to identify a enhancer which can be effective for skin penetration and nail penetration. The nail penetration and permeation studies showed that 10 % TU could be a potential enhancer for the nail permeation if the drug and the enhancer are to be incorporated in the same formulation. The skin permeation and penetration studies identified the effect of TU in increasing the levels of the drug in the epidermis while minimizing transdermal permeation.

From the data of saturated solubility and drug accumulation amount in nail clippings, it is also observed that the PEs which can enhance the solubility of EN to around 1mg/ml have the strongest ability to enhance the drug penetration amount into target tissues. Which can give the further experiment a good prediction that which PEs should be selected. A balanced approach in selecting PE which improve solubility and improve nail partitioning is favored. In this study an optimal drug solubility of 1 mg/ml in the PE was found.

In pH effect study, pH3.6; pH5 and pH7.4 PBS buffer are used. With a pH decrease, the uncharged form of EN will decrease which is beneficial to its solubility. TU was shown to improve the permeation beyond that of the lower pH. This was confirmed. pH 3.6 buffer was not studied because of possible irritation in subsequent formulations.

In drug-keratin binding study, the lower percentage of drug-keratin bound is favored because only free drug will be active against the causative organisms and also permeate through the nail plate into the skin tissue. In TU 10% pH7.4 buffer, there's significant drug binding to keratin which is 88.5%. This will immediately lead to the drop of the drug strength. By decreasing the pH to 5, the percentage decreased to 60% which is much preferred. This can be explained by with the pH decreases, the uncharged form of EN decreases, and in EN's two forms (charged and uncharged form), uncharged form has more keratin binding. Thus, as pH is decreased the binding of EN to keratin also decreases. This would product more free drug for diffusion.

In future study, a patch will be combined to this formulation, and how to create an environment of pH 5 in the patch will be studied.

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APPENDIX

A: Raw Data

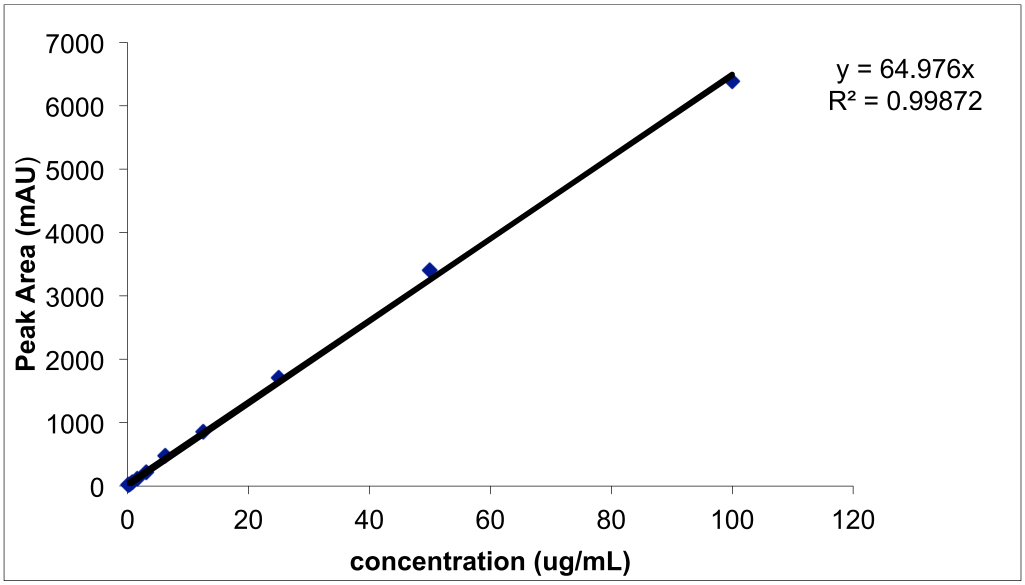

Figure A.1: Standard curve for econazole nitrate using HPLC

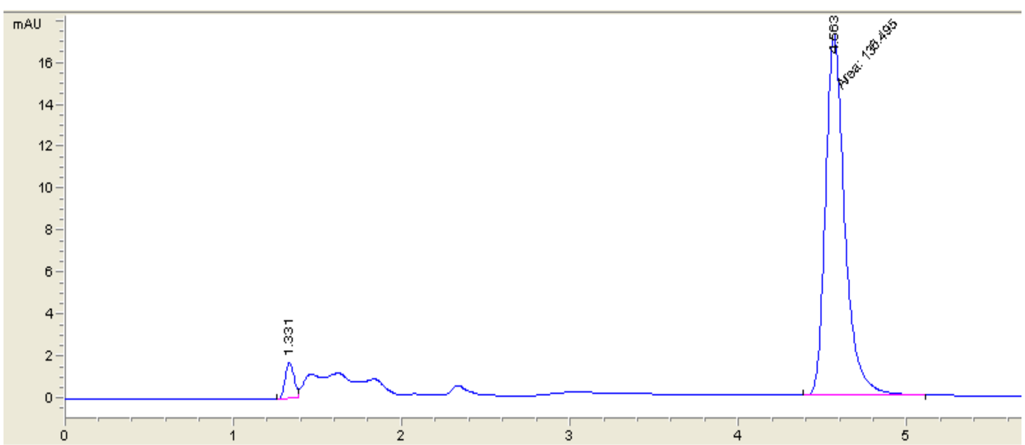

Figure A.2: HPLC Chromatogram of EN

B: Spread Sheet

$$J = \frac{dM}{Adt} = D\left(\frac{C1-C2}{t}\right) \ldots\ldots\ldots\ldots \text{(1.1)}$$

$$EF_{SOL} = \frac{EF_{PE}}{EF_{pH7.4PBS}} \ldots\ldots \text{(2.1)}$$

$$EFsim = \frac{EN_{simPE(\mu g/mg\ of\ nail)}}{EN_{sim\ pH7.4\ PBS(\mu g/mg\ of\ nail)}} \ldots\ldots \text{(2.2)}$$

$$EF_{flux} = \frac{Flux_{PE}\text{ of EN permeation }(\mu g/cm^2/hr)}{Flux_{\ pH\ 7.4\ PBS}\text{ of EN permeation }(\mu g/cm^2/hr)} \ldots\ldots \text{(2.3)}$$

$$\frac{EFepidermis}{EFdermis} = \frac{[EN]PE\ (\mu g/mL)\text{ in epidermis ordermis}}{[EN]\text{ pH 7.4 PBS }(\mu g/mL)\text{ in epidermis or dermis}} \ldots\ldots \text{(2.4)}$$