1.0. INTRODUCTION

The research topic chosen for the study is “Development, Formulation and Evaluation of Topical Preparations of Terbinafine Hydrochloride for the Treatment of Superficial Dermatophytosis”.

1.1. Topical drug delivery

Ointment, creams and gels are semisolid dosage forms intended for topical application. They may be applied to the skin, placed on the surface of the eye, or used nasally, vaginally or rectally. Topical preparations are used for both local and systemic effects. The following distinction is an important one with regard to dermatological applications. A topical dermatological product is designed to deliver drug into skin in treating dermal disorders, with the skin as target organ (Allen et al 2005).

Dermatological products applied to the skin are diverse in formulation and range in consistency from liquids to solid powders, but most popular products are semisolid preparations. Some of these may be non-medicated, in the sense that these may be devoid of any therapeutically active ingredients are used for cosmetic purposes (Jain NK 2006).
1.2. Skin (Remington et al 2004)

The skin often has been referred to as the largest organ of the body. Its accessibility and the opportunity it affords to maintain applied preparation intact for a prolonged time have resulted in its increasing use as a route of drug administration, whether for local, regional or systemic effects.

Anatomically, human skin may be described as a stratified organ with three distinct tissue layers: the epidermis, the dermis and the subcutaneous fat layer.

Epidermis the outermost skin layer comprises stratified squamous epithelial cells. Keratinized, flattened remnants of these actively dividing epidermal cells accumulate at the skin surface as a relatively thin region (about 10μm thick) termed the stratum corneum, or horny layer. The horny layer is itself lamellar with the keratinized cells overlapping one another, linked by intercellular bridges and compressed into about 15 layers. The lipid-rich intercellular space in the stratum corneum comprises lamellar matrices with alternating hydrophilic layers and lipophilic bilayers formed during the process of keratinization. The region behaves as a tough but flexible coherent membrane.
1.2.1. Dermis

The dermis apparently is a gel structure involving a fibrous protein matrix embedded in an amorphous, colloidal, ground substance. Protein, including collagen and elastin fibers, is oriented approximately parallel to the epidermis. The dermis supports and interacts with the epidermis, facilitating its conformation to underlying muscles and bones. Blood vessels, lymphatics, and nerves are found within the dermis, though only nerve fibers reach beyond the dermal ridges or papillae into the germinative region of the epidermis. Sweat glands and hair follicles extending from the dermis through the epidermis provides discontinues in an otherwise uniform integument.
The subcutaneous fat layer serves as a cushion for the dermis and epidermis. Collagenous fibers from the dermis thread between the accumulations of fat cells, providing a connection between the superficial skin layers and the subcutaneous layer.

1.2.2. Hair follicles and sweat glands

Human skins sprinkled liberally with surface openings extending well into the dermis. Hair follicles, together with the sebaceous glands that empty into the follicles, make up the pilosebaceous unit.

1.2.3. Pilosebaceous unit

Human hair consists of compacted keratinised cells formed by follicles. Sebaceous glands empty into the follicle site to form the pilosebaceous unit. The hair follicles are surrounded by sensory nerves; thus, an important function of human hair is sensory.

1.2.4. Sweat glands

Sweat glands are classified as apocrine and eccrine. Apocrine glands are secretory but are not necessarily responsive to thermal stimulation. Such glands do not produce sweat in the normal sense of the word. Apocrine glands, however often are associated with eccrine sweat glands, particularly in the axilla.
Eccrine sweat glands are coiled. Secretory glands, equipped with a blood supply, extending from the dermis to the epidermal surface. Eccrine sweat glands function to regulate heat exchange in human beings.

1.2.5. Drug effects and the extent of percutaneous drug delivery:

(Gennaro et al 2004)

Drugs are applied to the skin to elicit one or more of four general effects they are

i. An effect on the skin surface

ii. An effect within the stratum corneum

iii. More deep-seated effect requiring penetration into the epidermis and dermis.

iv. For a systemic effect resulting from delivery of sufficient drug through the epidermis and the dermis to the vasculature to produce therapeutic systemic concentration.

1.2.6. Percutaneous absorption:

Percutaneous absorption involves the transfer of drug from the skin surface to the stratum corneum, under the aegis of the concentration gradient, and its subsequent diffusion through the stratum corneum and underlying epidermis through the dermis and into the microcirculation. The skin behaves as a passive barrier to diffusing molecules. Evidence for this includes the fact that the impermeability of the skin persists long after the skin has been
excised. Furthermore, Fick’s law is obeyed in the vast majority of instances (Gennaro et al 2004)

Fick’s law of diffusion can be used to analyze the permeation data and can be used predicatively. Fick’s first law used to describe steady state diffusion and can be simplified to (Jain NK 2006)

\[ J = \frac{DK\Delta C}{h} \]

J = Flux per unit area

D = Diffusion coefficient

K = Skin vehicle partition coefficient

\( \Delta C \) = Concentration difference across the skin and ‘\( h \)’ is the diffusional path length

1.2.7. Factors affecting percutaneous absorption (Gennaro et al 2004)

- Skin hydration and temperature
- Penetration enhancers
- Stratum corneum and dermal clearance
- Cutaneous biotransformation.
1.3. Skin infections (Roxburgh’s et al 2003)

The stratum corneum is an excellent barrier to pathogenic microorganisms, but this itself sometimes the target of attack. The skin surface and its adherent structures harbour a stable microflora, which lives in symbiosis with skin and may indeed be beneficial. Gram positive cocci (Staphylococcus epidermidis), Gram positive lipophilic microaerophilic rods (Propionibacterium acnes) and Gram positive yeast like organism (Pityrosporum ovale or Malassezia furfur) live in the follicular lumina without normally causing much harm. However, under special conditions, excess sebum secretion, depressed immunity and compromised stratum corneum barrier protection can produce disease. Infection of the skin only occurs when the skin encounters a pathogen that its defences can not eliminate or control.

1.3.1. Fungal infections

The fungal infections of the skin are divided into superficial and deep fungal mycosis. Superficial fungal infections are dermatophytosis, tinea versicolor and candidiasis (Thappa DM 2005).

Most mycotic infections are superficial and limited to stratum corneum, hair and nails (James DW et al 2006).

The superficial mycoses contribute to a large extent in the incidence of dermatophytosis in any skin clinic in India, due to tropical climatic condition and have increased in recent years (Sawr GC 1985).
Terbinafine, an analogue of naftifine, is an allylamine antifungal agent used in the treatment of onychomycosis, tinea capitis and fungal skin infections (McClellan KJ et al 1999).

Terbinafine is similar to natifine 10-100 times more potent in vitro (Wolverton SE et al 2001).

Terbinafine is highly lipophilic, resulting in high concentration in and efficient binding to the stratum corneum, sebum and hair follicles, thus reducing the probability of reinfection (Wolverton SE. et al 2001).

For oral administration, Terbinafine hydrochloride 250mg and 500mg tablets are usually prescribed. Terbinafine is generally well tolerated after oral and topical administration (McClellan KJ et al 1999).

Terbinafine cream and topical solution 1% have shown efficacy in the treatment of tinea corporis, tinea cruris, tinea pedis, cutaneous candidiasis with mycological cure rates of 69-100% after four weeks of treatment (depending on the condition of the infection. (McClellan KJ et al 1999).

**1.3.2. Dermatophytosis**

Dermatophytes are related to fungi capable of causing skin changes of the type known as ringworm or dermatophytosis. Thus defined, the ringworm species are all moulds belonging to three asexual genera Microsporum, Trichophyton and Epidermophyton.

In the genus Microsporum, the macroconidia are rough, usually thick walled and range from fusiform to obovate in shape with 1-12 or more septa.
Those of *Trichophyton* species are thin walled, smooth and may be cylindrical, fusiform or clavate in shape, with upto 12 transverse septa. In *Epidermophyton*, the macroconidium is clavate, broadened and rounded at its distal pole, thin walled and has up to five septa; the conidia are smooth when first formed, but as the colony ages discrete are smooth when first may be observed.

**1.3.3. Clinical forms of ringworm infections**

The clinical features of dermatophyte infections result from a combination of keratin destruction and an inflammatory host response. The wide variation in clinical presentation depends upon the species and probably the strain of the fungus concerned, upon the size of the inoculum, upon the site of the body infected and upon the immune status of the host.

**1.3.4. Tinea infections (Sehgal VN. 2004)**

Tinea or ringworm infection is caused by a distinct class of fungi, the dermatophytes. They thrive in the keratin layer of the epidermis, nails and hair. However they do not invade the lining epidermis.

Dermatophytosis is caused by species of *Trichophyton*, *Microsporum* and *Epidermophyton*. The common species encountered in ringworm infection are as follows

*Microsporum audouni, Microsporum canis*

*Trichophyton rubrum, Trichophyton mentagrophytes,*
Trichophyton violaceum, Trichophyton tonsurans, Trichophyton verrucosum, Trichophyton schoenleinii

Epidermophyton floccosum.

Classification (Thappa DM 2005)

Clinically fungal infections are due to dermatophytes which are classified as follows depending upon

(tinea capitis), the bearded skin of the face (tinea barbae), the body (tinea corporis - mainly affecting children), the groin (tinea cruris or jock itch), the nails (tinea unguium), and the feet (tinea pedis or athlete's foot).

A. The site of involvements

The presence of tinea infection on the scalp, called tinea capitis.

Similarly the presence of tinea in the face, beard area, groin, hand, feet, body and nails are called as tinea faciei, tinea barbae, tinea cruris, tinea manuum, tinea pedis, tinea corporis and tinea unguium respectively.

B. Steroid modified tinea

If it is steroid modified then it is called as tinea incognito

C. Hypersensitivity to dermatophyte

If it is because of hypersensitivity reaction then it is called Dermatophytides.
**Tinea capitis** (Burns T et al 2004)

**Definition**: Ringworm of the scalp as shown in figure 2 in which the essential feature is invasion of hair shafts by dermatophyte fungus. Tinea capitis is predominantly an infection of children, although adult cases are seen particularly with Trichophyton tonsurens infections.

![Figure 2. Tinea capitis](image)

**Species concerned**: Most species of dermatophyte are capable of invading hair but some species (e.g. Microsporum audouinii, Trichophyton schoenleinii and Trichophyton violaceum) have a distinct predilection for the hair shaft.

**Tinea facei** (Burns T et al 2004)

**Definition**: Infection of the glabrous skin of the face with a dermatophyte fungus as shown in figure 3 (the moustache and beard areas of the adult male are excluded).

![Figure 3. Tinea facei](image)
Species concerned: Trichophyton mentagrophytes var. mentagrophytes and Trichophyton rubrum predominate but Microsporum audouinii and Microsporum canis are also common causes worldwide.

Tinea barbae (Burns T et al 2004)

Definition: Ringworm of the beard and moustache areas of the face with invasion of coarse hairs as shown in the figure 4. It is thus a disease of the adult male. Tinea of the chin and upper lip in females and children are considered as tinea faciei (ring-worm of the glabrous skin of the face).

Figure.4.Tinea barbae

Tinea cruris (Burns T et al 2004)

Definition: Infection of the groins by a species of dermatophytes as shown in the figure 5.

Figure.5.Tinea cruris
Species concerned: The causal species are those implicated in foot ringworm but in different proportions. Trichophyton rubrum is the main cause (Nerves H. et al 1960) mentagrophytes var. interdigitale and Epidermophyton floccosum also account for some cases.

Tinea manuum (Burns T et al 2004)

Definition: Any species of dermatophyte may affect the skin of the hand. Infections of the dorsal surface present no specific features and are considered as ringworm of the glabrous skin under the tinea corporis as shown in figure 6.

Figure 6. Tinea manuum

Species concerned: For the most part, the organisms concerned the three anthropophilic species involved in tinea pedos are Trichophyton rubrum

Tinea corporis (Burns T et al 2004)

Definition: Ringworm of the glabrous skin as shown in figure 7. The clinical manifestations result from invasion and proliferation of the causal fungi in the stratum corneum. Terminal hair in the affected parts may be invaded. By definition, it includes lesions of the trunk and limbs, excluding ringworm of specialized sites such as the scalp, feet and groins.
Species concerned. All known dermatophytes can produce lesions of the glabrous skin. Trichophyton rubrum is responsible for this condition. The animal species Trichophyton verrucosum and Trichophyton mentagrophytes var. mentagrophytes are responsible for the great majority of cases. (Devroey et al 1985). The anthropophilic species, Trichophyton violaceum, Trichophyton schoenleini, Trichophyton megnimmi and Trichophyton rubrum are recognized as occasional causes. (Ive FA et al 1968).

Tinea unguium (Burns T et al 2004)

Definition: Invasion of nail plates by species of dermatophytes as shown in figure 8. A different category of onychomycosis is associated with certain species of filamentous fungi that are frequently found in dystrophic nails; these are considered separately.
Species concerned: The Principal dermatophytes concerned are: i) with associated foot and hand infections- Trichophyton rubrum, Trichophyton mentagrophytes var.interdigitale and Epidermophyton floccosum; ii) with associated scalp infections- Trichophyton tonsurans, Trichophyton violaceum and Trichophyton soudanensei.

Tinea pedis (Burns T et al 2004)

Definition: Infection of the feet or toes with a dermatophyte fungus as shown in figure 9. The athelete’s foot is used by some to imply any form of cleft intertrigo.

Figure 9. Tinea pedis

Species concerned: Three anthropophilic species Trichophyton rubrum, Trichophyto mentagrophytes var.interdigitale and Epidermophyton floccosum together responsible for the vast majority of cases of foot ringworm throughout the world.
1.3.5. Diagnosis (Thappa DM 2005)

a. Wood’s light examination

Wood’s light is a low intensity ultraviolet light (340-400 nm) emitted by a high pressure mercury lamp fitted with a special filter made up of nickel oxide and silica. Observation of the tinea affected part of the skin under Wood’s light will show green fluorescence.

b. Potassium hydroxide examination of the skin

Potassium Hydroxide dissolves the keratin of keratinocytes, hairs and nails but does not dissolve the fungus.

Specimen from the skin is taken by using a 15 number scalpel blade and 10% potassium hydroxide solution in distilled water is to be added drop by drop and it is to be covered with cover slip. After 2-5 minutes, the excess potassium hydroxide is to be removed by filter paper and to be observed under microscope. Dermatophytes appear as septate branching hyphae in the scales or hyphae and spores in hair shaft.

Figure.10. Potassium hydroxide examination of the skin
Tinea infection (ringworm) caused by the species of Trichophyton, Microsporum and Epidermophyton species of fungus and is restricted to the stratum corneum, the hairs and nails. Diagnosis is confirmed by identifying the fungi in the scales by direct microscopy and culture. Treatments with topical imidazole, topical Terbinafine or oral Terbinafine are most suitable (Marks et al 2003)

1.4. Rationale for topical drug delivery (Jain NK 2006)

There are three fundamental approaches by which the biopharmaceutical problems of formulating a successful topical dosage forms are solved. They are

i. First approach is to assist or manipulate the barrier functions of the skin.

ii. Second approach is to breach the horny layer at the molecular scale so as to direct drugs to the viable epidermal and dermal tissues without using oral, systemic or other therapies.

iii. The third approach is to use the skin deliberately as a portal entry into the systemic circulation.

1.4.1. Dermatological formulation

Dermatological formulations are used to treat condition and diseases of the skin. It includes ointments, creams, gels, and pastes. Other dosage forms include solution, powders and transdermal drug delivery systems (Aulton et al 2004).
In treating skin diseases, the drug in a medicated application should penetrate and retained in the skin for a while. Drug penetration into the skin depends on a number of factors, including the physiochemical properties of the medicated substance, the characteristics of the pharmaceutical vehicle, and the condition of skin. (Allen et al 2005).

1.4.2. Physiochemical criteria for dermatological formulations

The developer of dosage form must note the physical and chemical behavior of the drug and the dosage form during preformulation studies, bench-scale work, pilot studies and batch processing, at the manufacturing level, and during storage and use of a product.

Some general factors must be considered during the development of new semisolid dosage forms and storage that includes

i. Stability of active ingredient

ii. Stability of adjuvants

iii. Rheological properties – consistency, viscoelasticity, extrudability

iv. Loss of volatiles, including water

v. Phase changes – in homogeneity, cracking

vi. Particle size distribution of dispersed phase

vii. Apparent pH

viii. Particulate contamination
1.5. **Preformulation studies** (Cartesen JF et al 2003)

The goals of the preformulation studies are

To establish the necessary physiochemical parameters of new drug substance.

To establish its physical characteristics.

To establish its compatibility with common excipients.

Physiochemical studies are usually associated with greater precision and accuracy, and in the case of new drug substance would include studies of (a) pKa b) solubility c) melting point and polymorphism d) vapor pressure e) surface characteristics (surface area, particle shape, pore volume) and f) hygroscopicity.

1.5.1. **Compatibility studies** (Cartesen JF et al 2003)

Prior to attempting the first formulation with a new drug, most research groups carry out compatibility testing the principle is to make up reasonably rationed mixtures of drug and excipient, to ascertain which excipients may be reasonably used in with the drug. The methods used nowadays have followed in step with analytical developments and are a) chemical assay b) TLC c) HPLC d) DSC e) FT – IR f) micro calorimetric materials.
1.6. Ointments

Ointments are greasy, semisolid preparation, often anhydrous and containing dissolved or dispersed medicaments. Hydrocarbon bases are usually consisting of soft paraffin or mixtures with hard paraffin. Paraffin forms a greasy film on the skin, inhibiting moisture loss and improving hydration of the horny layer in dry scale condition. This hydration is also a main reason for ointments are so effective in encouraging percutaneous absorption of a drug.

The plastibases are a series of hydrocarbon containing polyethylene, which forms a structural matrix in system, which are fluid at the molecular scale but are typical dermatological semisolids. They are soft, smooth, homogenous, neutral colourless, odourless, non-irritating, non-sensitizing, extremely stable vehicles. Plastibases are compatible with most medicaments and they maintain their consistency even at high concentration of solids and under extremes of temperature.

The bases apply easily and spread readily, adhere to the skin, importing a velvety, non-greasy feel, and can readily be removed.

Fat and fixed oil bases are dermatological vehicles have frequently contained fixed oils of vegetable origin, consisting essentially of the mono, di- and triglycerides of mixtures of saturated and unsaturated fatty acids. The most common oil includes peanut, sesame, olive, cottonseed, almond and arachis oil. Such oils decompose an exposure to air, light and high temperature, and may turn rancid.
1.6.1. Bases

1.6.1.1. Absorption bases

Absorption bases soak up water to form water in oil emulsions while retaining their semisolid consistencies. Eventually they are anhydrous vehicles composed of a hydrocarbon base and a miscible substance with polar groups that function as a water-in-oil emulsifier, e.g. lanolin, cholesterol and other sterols and, esters of polyhydric alcohols such as sorbitan monostearate or mono-oleate. They deposit greasy film on the skin but suppress less the transepidermal water loss. Some individuals are sensitive to lanolin.

1.6.1.2. Emulsifying bases

These essentially anhydrous bases contain oil-in-water emulsifying agents, which make them miscible with water and so washable or ‘self-emulsifying’. These are three types, depending on the ionic nature of the water – soluble emulsifying agents. They are anionic, cationic & non-ionic. The bases mix with aqueous secretions and readily wash off the skin; thus they are useful for scalp treatments.

1.6.1.3. Water – Soluble bases

It is a combination of high and low molecular weight polyethylene glycols (Macrogols, carbowaxes). Suitable combinations provide products with an ointment like consistency, which soften or melt on skin application. They are non-occlusive, mix readily with skin exudates and do not stain cloths.
The macrogols do not hydrolyse, detoriate, support mould growth or irritate the skin.

1.7. Method of preparation of ointments (Ansel et al 2005)

Ointments are prepared by two general methods a) incorporation and b) fusion depending primarily on the nature of the ingredients.

1.7.1. Incorporation

On a small scale, as in extemporaneous compounding, the pharmacist may mix the component using a mortar and paste, or a spatula may be used to rub the ingredients together on an ointment slab.

The ointment base is placed on one side of the working surface and the powdered components previously reduced to fine powders and thoroughly blended in a mortar, on the other side. A small portion of the powder is mixed with a portion of the base until uniform. Geometric dilution is continued until all portions of the powder and base are combined and thoroughly and uniformly blended.

1.7.2. Fusion

By the fusion method, all or some of the components of an ointment are combined by being melted together and cooled with constant stirring until congealed. Components not melted are added to the congealing mixture as it is being cooled and stirred. On small scale, fusion may be conducted in a
A porcelain dish or glass beaker. On large scale, it is carried out in large steam jacketed kettles.


Creams are semisolid emulsion for external application. Oil in water emulsions are most useful as water washable bases, whereas water-in-oil emulsion are emollient and cleansing purpose.

Creams find primary application in topical skin products. Many patients and physicians cream to ointments because they are easier to spread and remove. (Allen et al 2005).

Creams are emulsions for external use. The emulsion typically contain globules ranging from 0.1 to 100 nm in diameter.

1.8.1. Stability of emulsion

There are two principal requirements to ensure the stability of emulsion. First, there should be no appreciable change in either the mean particle size or the size distribution of droplets of the dispense phase throughout the shelf life of the product. Second, there should be a homogenous distribution of the emulsified droplets throughout the system. (Waltur 1994).
1.8.2. Emulsifying agents (Allen et al 2005)

The initial step in preparation of an emulsion is a selection of the emulsion. To be useful in a pharmaceutical preparation, the emulsifying agent must be compatible with the other formulative ingredients and must not interfere with the stability or efficacy of the therapeutic agent.

Various types of materials have been used in pharmacy an emulsifying agents which includes the following.

1. Carbohydrate materials, such as the naturally occurring agent’s acacia, tragacanth, agar and pectin.

2. Protein substance, such as gelatin, egg yolk and casein.

3. High molecular weight alcohols such as stearyl alcohol, cetyl alcohol and glycerol mono stearate.

4. Wetting agents includes sorbiton esters and polyoxy ethylene derivatives.

5. Finely divided solids such as colloidal clays, including bentonite, magnesium hydroxide and aluminium hydroxide.

1.8.3. HLB System (Allen et al 2005)

Generally, each emulsifying agent has a hydrophilic and lipophilic portion, with one or the other being, more or less predominant and influencing in the manner already described the type of emulsion. Although the numbers of HLB value have been assigned up to about 40, the usual range between 1 and
20. Materials that are highly polar or hydrophilic have been assigned higher numbers than materials that are less polar and more lipophilic. Generally, surface active agents having an assigned HLB value of 3 to 6 are greatly lipophilic and produce water in oil emulsion and agents with HLB values of about 8 to 18 produce oil in water emulsions.


Emulsions are creams that may be prepared by several methods, depending upon the nature of the components and the equipment. On a small scale, as in the laboratory or pharmacy, emulsion may be prepared by using a dry wedge wood or porcelain mortar and pestle, mechanical blenders or mixer, a hand homogenizer, a bench-type homogenizer, or sometimes a simple prescription bottle.

On a large scale, large mixing tanks may be used to form the emulsion through the action of a high-speed impeller.

In small scale extemporaneous preparation of emulsions, three methods may be used, they are the continental or dry gum method, the English or wet gum method, and the bottle or Forbes bottle method. In the first method, the emulsifying agent (usually acacia) is mixed with the oil before the addition of water. In the second method, the emulsifying agent is added to the water (in which it is soluble) to form mucilage, and then the oil is slowly incorporated to form the emulsion. The bottle method is reserved for volatile oils or less viscous oils and is a variation of the dry gum method.
1.10. Gels (Allen et al 2005)

Gels are defined as semisolid systems consisting of dispersions made up of either small inorganic particles or large organic molecules enclosing and interpenetrated by a liquid.

Gels are also defined as semi rigid system in which the movement of the dispensing medium is restricted by an interlacing three-dimensional network of pesticides or solvated macromolecules of the dispensed phase. A high degree of physical or chemical cross-linking may be involved.

The increased viscosity caused by the interlacing and consequential internal friction is responsible for semisolid state.

Gel systems are as clear as water, grid others are turbid, since the ingredients may not be completely molecularly disperse (soluble or insoluble), or they may form aggregates, which disperse light. The concentration of the gelling agents is mostly less than 10% usually in 0.5 to 2.0% range, with some excipients.
1.10.1. Classification and types of gels (Allen et al 2005)

Table 1. Classification and types of gels

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Examples</th>
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</thead>
<tbody>
<tr>
<td>Inorganic</td>
<td>Two phase system</td>
<td>Aluminium hydroxide gel</td>
</tr>
<tr>
<td>Organic</td>
<td>Single phase system</td>
<td>Carbopol</td>
</tr>
<tr>
<td>Hydro gel</td>
<td>Organic hydro gels natural and synthetic gums</td>
<td>Pectin paste, bentonite gel</td>
</tr>
<tr>
<td></td>
<td>inorganic hydro gels</td>
<td></td>
</tr>
<tr>
<td>Organo gel</td>
<td>Hydrocarbon type</td>
<td>Petrolatum, cocoa butter</td>
</tr>
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</table>

1.10.2. Gelling agents (Allen et al 2005)

Gelling agents include acacia, alginic acid, bentonite, carbomer, carboxymethyl cellulose sodium, ceto stearyl alcohol, colloidal silicon dioxide, ethylcellulose, gelatin, guar gum, hydroxyl propyl methyl cellulose, magnesium aluminium silicate, maltodextrin, methyl cellulose, polyvinyl alcohol, povidone, propylene carbonate, sodium alginate, sodium starch glycollate, starch, tragacanth, and xanthan gum. A few more includes alginic acid and carbomer.

Carbomer resins and high – molecular weight allyl pentaerythritol – cross – linked acrylic acid – based polymers modified with (10 to 30 alkyl acrylates. These are many carbomer resins, with viscosity ranges from 0 to 80000 cps, carbomers are 910, 934, 934P, 940, 1342 are official in USP-26-
NF21. Carbopol 940 forms a sparkling clear water or hydro alcoholic gels. (Allen et al 2005)


Gels are prepared by freshly preparing the disperse phase to achieve a fine degree of subdivision of particles and a gelatinous character to those particles. The desired gelatinous precipitate results when solutions of inorganic agents react to form an insoluble chemical having a high attraction for water. As the microcrystalline particles of the precipitate develop, they strongly attract water to yield gelatinous particles, which combine to form the desired gelatinous precipitate. In addition to the water vehicle, other agents as propylene glycol, propyl gallate and hydroxy propyl cellulose may be used to enhance gel formation.

1.12. Evaluation of topical formulations

A good disperse system formulation should have both physical and chemical stability and cosmetic acceptability. Disperse systems can vary in appearance and full due to viscosity, glass, smoothness and texture. (H.A Libermann et al 2004)

1.12.1. Viscosity

It is defined on the fluid resistance to change in form due to internal friction. Methods used for absolute viscosity measurements are flow through a tube, rotational methods or surface viscosity methods. Methods used for
relative viscosity measurement are those using orifice viscometer, falling balls, or plungers. (Libermann HA et al 2004)

1.12.2. Particle size distribution

Fine droplets or particles are described in terms of concentration, size and size distribution. The particle size is the disperse system depends on both the method of manufacture and formula used. The size of the droplet or particle can affect product appearance. (Libermann HA et al 2004)

1.12.3. Electrical conductivity

This can be determined by measuring zeta potential. Zeta potential is the work required to bring a unit charge from infinity to the edge of a fixed interface layer. Since the stability of an emulsion is so much a function of the electrical properties of the interface (Libermann, et al 2004)

1.12.4. Determination of disperse system type

It is used to characterize the type of emulsion. This can be determined by dilution, solubility and conductivity method. (Libermann, et al 2004)

1.12.5. Texture analysis

Mechanical properties were examined using a texture analyzer (TA-XT2). Texture analyzers are used to detect consistency, stickiness, firmness hardness of the formulations.
1.12.6. Extrudability

It is the one of the physical test to evaluate the force required to extrude the contents from either tube or sachet style packing.

1.12.7. Rheological studies

The topical formulations are subjected to rheological studies to access their flow behavior as quality control measure.

1.12.8. Chemical evaluation

Chemical evaluation includes

- Content uniformity test
- pH measurement

Content of uniformity of the topical semisolids can be decolorised by suitable calorimetric or spectrophotometric methods.

Change in the pH of the product indicates the chemical decomposition most probably of a hydrolytic nature (Jain NK 2006).


The permeation rate of the drug across skin has been measured using several different kinds of in-vitro skin permeation apparatus. A typical apparatus has three main compartments; they are donor, membrane and receptor compartment.
The physiological saline or phosphate buffer solution maintained at 37°C in the receptor compartment. This will keep the skin surface approximately 32°C, which simulates the temperature of the human skin. The samples were analysed in specified time intervals to determine the release of the drug from the membrane. Different types of permeation apparatus include Franz diffusion cell and the flow through diffusion cell.

1.13. Packaging of semisolids

Most semisolid products are manufactured by heating and are filled into the container while cooling still in the liquid state.

Topical dermatological products are packed in either jar or tubes whereas ophthalmic, nasal, vaginal and rectal semisolids are always packed in tubes.

Ointment tubes are made up of aluminium or plastic. Ointment, cream and gels are most frequently packed in 5, 15, and 30g tubes.

1.14. Stability testing as per ICH guidelines


The aim of the stability testing is to ensure the quality, safety and efficacy of drug products up to their expiration date. This means that all organoleptic, physiochemical, chemical and microbial test results must be within the shelf life tolerance ranges up to the end of the shelf life.

Stability of the pharmaceutical preparation can be defined as “the capacity of a particular formulation (dosage form or drug product) in a specific
container / closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications throughout in shelf life”. Stability testing has become an integral part of formulation development. It is also a part of dossier submission to regulatory agencies for licensing approval.

Factors affecting stability:

1) Storage time  
2) Storage conditions  
3) Type of dosage form  
4) Container and closure system

1.14.2. Generation of stability data

Stability data generation is the documentation of a detailed protocol. The protocol depends on type of drug and dosage form, proposed container – closure system and on the target market and its average temperature and humidity. A properly designed protocol contains the following information.

i. Type, size and number of batches

ii. Plan of sampling

iii. Storage conditions

1.14.3. Test parameters for topical preparations

a. Appearance and clarity  
d. pH

b. Color and odor  
e. Viscosity

c. Homogeneity  
f. Particle size
1.15. **Topical antifungal agents** (Wolverton SE et al 2001)

Fungal infections are among the most common diseases of the skin and are second to acne as the most common condition treated by dermatologists. Topical antifungals are generally considered as first-line therapy for uncomplicated, superficial, relatively localized dermatomycoses due to their high efficacy and low potential for systemic adverse effects.

Today there are multiple modern topical anti-mycotics capable of achieving clinical and mycologic eradication of human dermatomycoses. The most commonly employed topical antifungals are among three main classes; the polyenes, the azoles, and the allyl amines / benzyl amines. Other topical anti-mycotics (not among these major drug classes) include a hydroxypyridone (ciclopirox olamine), selenium sulfide, and a thiocarbonate (tolnaftate).

1.15.1. Polyenes

Developed in the late 1950s, polyene antimycotics were the first agents to have specific antifungal properties. Polyene antifungal are characterized by a macrolide ring of carbon atoms containing a number of conjugated double bonds (-C=C-C=C-), hence the name “polyene”. The polyene macrolide ring is closed by an internal ester or lactose. The two clinically significant and readily available polyenes are nystatin and amphotericin B.

1.15.2. Azoles

The introduction of theazole antimycotics presented a new class of compounds with a broader spectrum of activity, including activity against the
common dermatophytes that are not susceptible to the polyenes. Azoles act by blocking the biosynthesis of ergosterol, the primary sterol derivative of the fungal cell membrane.

Depletion of ergosterol results in membrane permeability changes incompatible with fungal growth and survival. The azoles block sterol synthesis by interfering with the cytochrome P-450 dependent enzyme, lanosterol 14a–demethylase, which catalyzes the conversion of lanosterol or ergosterol. The binding of the azoles proceeds primarily by a direct link of an azole nitrogen to the heme iron located in a binding domain of the cytochrome P-450 molecule.

1.15.3. Allyl amines and Benzyl amines

The allylamines represent a newer class of anti-mycotic agents. Allylamines are broad-spectrum antimycotics with both fungistatic and fungicidal activity (depending on the organism being tested) that act by inhibiting the synthesis of ergosterol, an essential component of the fungal cell membrane. Such inhibition leads to cell membrane fragility, increased membrane permeability, and intracellular accumulation of sterol precursors. The allylamines act at an earlier step in the ergosterol biosynthesis pathway than the azole class of antifungal drugs, and their inhibition is cytochrome P-450-independent. Natifine and terbinafine are the two primary antimycotics of the class of allylamines. Butenafine is the first and only representative of the benzyl amine class, a group similar in structure and action to the allylamines.
1.15.4. Naftifine

Naftifine is a synthetic allyl amine antifungal with the chemical structure (E) – N-methyl –N- (1-naphthylmethyl)-3-phenyl-2-propen-1-amine-hydrochloride.

Mechanism of action

The action of naftifine is both fungicidal and fungistatic. Naftifine inhibits squalene epoxidase, the enzyme responsible for the conversion of squalene to squalene oxide in the ergosterol biosynthesis pathway. With this interruption, there is decreased ergosterol production and increased accumulation of the sterol precursor squalene. Ergosterol is an essential component of the fungal cell membrane and necessary for fungal growth and survival. Studies show that the fungicidal activity of naftifine results from interference with ergosterol synthesis, accumulation of squalene, and consequent disruption of fungal cell membranes. The inhibitory action of naftifine differs from the action ofazole antifungals, with its inhibitory effect being independent of the cytochrome P-450-dependent synthesis of steroidal hormones.

1.15.5. Terbinafine

Terbinafine (E – N - 6, 6 - dimethyl – 2 - hepten-4 – ynyl ) - N - methyl-1-naphthalena-methanamine) is a broad-spectrum synthetic anti-mycotic agent of the allyl amine chemical family with both fungistatic and fungicidal properties.
Absorption

Terbinafine is a highly lipophilic, resulting in high concentration in and efficient binding to the stratum corneum, sebum, and hair follicles, thus reducing the probability of reinfection. Pharmacokinetic studies demonstrated persistent concentrations well above the MICs for the common dermatophytes 7 days after topical application. Terbinafine is an allyl amine derivative similar to naftifine but 10 to 100 times more potent in vitro.

Mechanism of action

Terbinafine exhibits a broad antifungal spectrum that interferes with the ability of squalene epoxidase to catalyze the conversion of squalene to ergosterol. The suppression of biosynthesis of ergosterol, a sterol critical to cellular integrity, and the intracellular squalene accumulation result in cell death. Terbinafine inhibits ergosterol biosynthesis at an earlier stage than theazole antifungals, without affecting cytochrome P-450-related steroidogenesis. This earlier mode of action may account for its fungicidal rather than fungistatic activity.

1.15.6. Ciclopirox olamine

Ciclopirox olamine (6-cyclohexyl-1-hydroxy-4-methyl-2 (1H) – Pyridone ethanolamine) is a hydroxypyridone antifungal agent with a unique structure and a mode of action unrelated to the other available antifungals.
1.15.7. Selenium Sulfide

Selenium sulfide is a liquid anti-seborrheic, antifungal preparation for topical application only. It is available in a prescription only 2.5% lotion and in a 1% lotion (Selsun Blue), which is available over the counter.

1.15.8. Undecylenic Acid

Undecylenic acid preparations consist of the compound undecylenic acid and its zinc, calcium, or sodium salt in a powder, aerosol, cream, or solution. It is used in the treatment of various dermatomycoses, including tinea pedis, tinea cruris, and diaper dermatitis.

1.16. Content analysis by using High pressure liquid chromatography (Satinder A et al 2005)

HPLC provides reliable quantitative precision and accuracy, along with a linear dynamic range (LDR) sufficient to allow for the determination of the API and related substances in the same run using a variety of detectors, and can be performed on fully automated instrumentation. HPLC provides excellent reproducibility and is applicable to a wide array of compound types by judicious choice of HPLC column chemistry. Major modes of HPLC include reversed phase and normal phase for the analysis of small (< 2000 Da) organic molecules, ion chromatography for the analysis of ions, size exclusion chromatography for the separation of polymers, and chiral HPLC for the determination of enantiomeric purity.
In normal-phase HPLC, solute retention is based on the distribution of solute between a polar stationary phase and a nonpolar mobile phase (typically a mixture of hexane and a more polar solvent such as isopropanol). Elution may be promoted by increasing the amount of polar solvent in the mobile phase. In reversed-phase HPLC, retention is based on distribution between a non-polar stationary phase and a polar mobile phase (typically a mixture of water and acetonitrile or methanol), and elution, is promoted by addition of the less polar solvent to the mobile phase. With the exception of extremely polar or ionized compounds, which are not amenable to normal-phase HPLC, and extremely nonpolar compounds such as certain steroids and natural products, which are not amenable to APIs and related substances. However, about 75% of current HPLC analyses are performed using the reversed-phase. This is due not only to safety considerations using nonpolar solvents but also to the differences in sample preparation procedures required for normal-phase versus reversed-phase HPLC.

In summary, HPLC, particularly reversed-phase HPLC, is currently the most suitable method for meeting most of the criteria for quantitative analysis within the pharmaceutical industry.


1.16.1.1 Apparatus

A liquid chromatograph consists of a reservoir containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic
column, a detector, and a data collection device such as a computer, integrator, a recorder, and a data collection device such as a computer, integrator, or recorder. Short, small-bore columns containing densely packed of compounds or stationary phase provide for the rapid exchange of compounds between the mobile and stationary phases. In addition to receiving and reporting detector output, computers are used to control chromatographic settings and operations, thus providing for long periods of unattended operation.

1.16.1.2. Pumping systems

HPLC pumping systems deliver metered amounts of mobile phase from the solvent reservoirs to the column through high-pressure tubing and fittings. Modern systems consist of one or more computer-controlled metering pumps that can be programmed to vary the ratio of mobile phase components, as is required for gradient chromatography, or to mix isocratic mobile phases (i.e. mobile phases having a fixed ratio of solvents). However, the proportion of ingredients in premixed isocratic mobile phases can be more accurately controlled than in those delivered by most pumping systems.

1.16.1.3. Injectors

After dissolution in mobile phase or other suitable solution, compounds to be analyzed are injected into the mobile phase, either manually by syringe or loop injectors, or automatically by auto samplers. The latter consist of a carousel or rack to hold sample vials with tops that have a pierceable septum or stopper and an injection device to transfer sample from the vials to a loop from which it is loaded into the chromatograph.
A syringe can be used for manual injection of samples through a septum when column head pressures are less than 70 atmospheres (about 1000 psi). At higher pressures an injection valve is essential. Some valve systems incorporate a calibrated loop that is filled with test solution for transfer to the column in the mobile phase.

1.16.1.4. Columns

For most pharmaceutical analyses, separation is achieved by partition of compounds in the test solution between the mobile and stationary phases. A system consisting of polar stationary phases and nonpolar mobile phases are described as normal phase, while the opposite arrangement, polar mobile phases and nonpolar stationary phases, is called reverse-phase chromatography.

Stationary phases for modern, reverse-phase liquid chromatography typically consist of an organic phase chemically bound to silica or other materials. Particles are usually 3 to 10mm in diameter, but sizes may range up to 50mm or more for preparative columns. Small particles thinly coated with organic phase provide for low mass transfer resistance and hence rapid transfer of compounds between the stationary and mobile phases. Column polarity depends on the polarity of the bound functional groups, which range from relatively nonpolar octadecyl silane to very polar nitrile groups. Liquid, nonbound stationary phases must be largely immiscible in the mobile phase. Even so, it is usually necessary to presaturate the mobile phase with stationary phase to prevent stripping of the stationary phase from the column. Polymeric stationary phases coated on the support are more durable.
Columns used for analytical separations usually have internal diameters of 2 to 5mm; larger diameter columns are used for preparative chromatography. Columns may be heated to give more efficient separations, but only rarely are they used at temperatures above 60°C because of potential stationary phase degradation or mobile phase volatility. Unless otherwise specified in the individual monograph, columns are used at ambient temperature.

1.16.1.5. Detectors

Many compendial HPLC methods require the use of spectrophotometric detectors. Such a detector consists of a flow-through cell mounted at the end of the column. A beam of ultraviolet radiation passes through the flow cell and into the detector. As compounds elute from the column, they pass through the cell and absorb the radiation, resulting in measurable energy level changes.

Fixed, variable and multi-wavelength detectors are widely available. Fixed wavelength detectors operate at a single wavelength, typically 254nm, emitted by a low-pressure mercury lamp. Variable wavelength detectors contain a continuous source, such as a deuterium or high-pressure xenon lamp, and a monochromator or an interference filter to generate monochromatic radiation of a wavelength selected by the operator. Modern variable wavelength detectors can be programmed to change wavelength while an analysis is in progress. Multi-length detectors measure absorbance at two or more wavelengths simultaneously.
1.16.1.6. Data-collection devices

Modern data stations receive and store detector output and print out chromatograms complete with peak heights, peak areas, sample identification and method variables. They are also used to program the liquid chromatograph, controlling most variables and providing for long periods of unattended operation (USP 24 NF -19 2000).
1.17. Objective of the Present Study

Dermatophytosis (also known as ringworm or tinea) is a superficial fungal infection of the skin, hair or nails. This group of superficial fungal infections is usually classified according to location on the body.

Dermatophytosis (tinea) may affect the scalp (tinea capitis), the bearded skin of the face (tinea barbae), the body (tinea corporis - mainly affecting children), the groin (tinea cruris or jock itch), the nails (tinea unguium), and the feet (tinea pedis or athlete's foot). These disorders vary from mild inflammations to acute vesicular reactions.

Although remissions and exacerbations are common, with effective treatment, the cure rate is very high. The treatment needs effective cure. Currently cream formulation of Terbinafine hydrochloride only is available. It may not be suitable for all varieties of skin conditions namely dry skin, wet skin and normal skin.

The objective of the study is to formulate and evaluate the efficacy of topical preparations of Terbinafine hydrochloride for the treatment of various types of superficial dermatophytosis. 1% Terbinafine hydrochloride cream is available in the market. However ointment and gel formulations of Terbinafine hydrochloride are not available in the market. Hence it is decided to formulate a stable 1% Terbinafine hydrochloride ointment, cream and gel and to find out their clinical efficacy and compare the efficacy.
The study is divided into:

i) Development of several topical preparations of Terbinafine hydrochloride as ointment, gel and cream.

ii) Choosing one best formulation each of ointment, cream and gel based on physiochemical evaluations which include appearance, color, pH, rheological character, spreadability, extrudability, firmness, consistency, cohesiveness, hardness and stickiness. For the estimation of drug content, a more sensitive HPLC method is used.

iii) In-vitro diffusion study of the best formulations by using Franz diffusion cell.

iv) Stability study of the best formulations as per ICH guidelines.

v) Assessment of comparative clinical efficacy of the best formulations and a marketed cream formulation in patients with superficial dermatophytosis at Department of Dermatology, Sri Ramachandra Hospital, Porur, Chennai-600 116, India.