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Research Article

PREPARATION AND EVALUATION OF TERBINAFINE NANOEMULGEL FOR FUNGAL INFECTION

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Abstract:

Objective: Terbinafine hydrochloride was BCS class 2 drug which has low aqueous solubility, so for increase their antifungal activity their solubility improvement is necessary.

Methods: High energy emulsification method was used for preparation of nano-emulgel. By using probe sonicator, ultrasonic waves was generate for decreasing proper particle size, without degrade their own structure.

Results: In preparation of the nanoemulsion (NE), excipients were selected based on the solubility study. Capryol oil was optimized as the oil phase. Tween 80 and propylene glycol were optimized as the surfactant and co-solvent respectively, and were mixed (Smix) in 1:3 weight ratios. The optimum nanoemulsion formulae containing 10 or 15% w/w oil, 45% w/w Smix (1:3) and 45-40% w/w aqueous phase) were incorporated into Carbopol 940 gel based emulgel formulae.

Conclusion: Terbinafine Hydrochloride is an allylamine antifungal drug widely used in the treatment of infections caused by dermatophytes, and their activity was increased by preparing nanoemulgel for industrial application in the future and therapeutic needs.

Keywords: Nano-emulsion, surfactant, fungal diseases, infection, emulgel, transdermal drug delivery system, penetration

Introduction[1,2]:

Emulgels are either emulsion of oil in water or water in oil type, which is gelled by mixing it with gelling agent. Incorporation of emulsion into gel increases its stability & makes it a dual control release system. Due

to lack of excess oily bases & insoluble excipients, it shows better drug release as compared to other topical drug delivery system. Presence of gel phase makes it a non-greasy & favours good patient compliance.

Fungal Diseases: Superficial infections are confined to skin, hair, nails or mucous membranes. The most common fungal skin infections are the dermatophytoses, pityriasisversi colour, and candidiasis. Approximately 90% of fungal skin infections are caused by 'dermatophytes', which are parasitic fungi affecting the skin, hair, nails.

- It is also reported to have good activity in vitro against *Cryptococcus*, some species of *Candida*, *Penicillium marneffei*, *Aspergillus*, and other filamentous fungi.

-The mode of action for terbinafine Hydrochloride involves inhibition of enzyme squalene epoxidase in fungal ergosterol biosynthesis, which induces accumulation of intracellular squalene and cells death.

- Topical therapy is an attractive choice for the treatment of the cutaneous infections due to its advantages such as targeting of drugs to the site of infection and reduction of the risk of systemic side effects.

- Systemic treatment is usually reserved for infections of the nails, extensive cutaneous infections or those which have not responded to topical therapy. --Conventional topical formulations are unable to retain the drug over the skin for a prolonged period and hence necessitate longer treatment duration or have to be supplemented by oral therapy.

- For effective local delivery of an antifungal that is applied to the surface of the skin, the agent must be partitioned firstly from the vehicle into the stratum corneum, and then partitioned to the local tissues including the viable epidermis, dermis, subcutaneous tissue and appendages.

- The need for multiple applications a day is frequently associated with poor compliance of patients. Thus, prolonging the contact time of active substances to the skin and thereby reducing the application frequency is subject of intensive research.

- Sustained release delivery systems with features of both semisolid formulations and patches may be employed here. The concept of film forming formulations is very recent.

-Film forming formulations may be solutions, gels or emulsions. Film forming formulations are defined as non-solid dosage forms that produce a substantial film in situ after application on the skin or any other body surface.

- Such compositions can either be liquids or semisolids with a film forming polymer as basic material for the matrix. The formed film is sufficiently substantial to provide a sustained drug release to the skin.

Transdermal drug delivery system[2]

Transdermal drug delivery system is a therapeutic system of defined surface area that delivers a predetermined amount of drug to the surface of intact skin at a pre-programmed rate. These systems systemically provide drug at a predictable rate and maintain the rate for extended period of time, thus eliminating numerous problems associated with oral products such as, unpredictable or reduced bioavailability, enhanced first pass hepatic metabolism, relatively short residence time, dose dumping and dose inflexibility. Also, transdermal drug delivery system provides continuous percutaneous administration of a drug at controlled rate which permits elimination of pulse entry into the systemic circulation, a phenomenon often associated with side effects. It also allows the option of rapidly terminating absorption of medication therapy, which needs to be interrupted. Hence these systems can be designed to put in drugs, through intact skin at appropriate rates to maintain suitable plasma drug levels for therapeutic efficacy, without periodic fluctuations into plasma concentrations that would accompany toxicity or lack of efficacy. Throughout first half of the twentieth century many advances were made with regards to understanding of topical drug delivery for local effect. The findings accumulated over the years practically revolutionized the old theory of an impermeable skin barrier and motivated a number of researchers to develop rate controlled drug delivery system for controlling the transdermal administration of drugs to accomplish the objective of systemic medication. Hence in case of transdermal drug delivery system skin becomes the main route of administration with large surface area covering the body, layered anatomy and defined physicochemical properties.

Anatomy of skin

The skin is multilayered organ composed of many histological layers. It is generally described in terms of three tissue layers. Skin is an anatomical barrier between the body and its environment and contributes to 16-18 % of normal body weight.

1. Epidermis: the outermost skin layer comprises of stratified squamous epithelial cells. The epithelial cells are held together mainly by highly convoluted interlocking bridges, which are responsible for the unique integrity of the skin. Microscopic sections of the epidermis show two main parts: the stratum corneum and

the stratum germinativum. The stratum corneum forms the outermost layer of the epidermis and consists of many layers of compacted flattened.They are formed and continuously replenished by the slow upward migration of cells produced by basal cell layer of the stratum germinativum the transition from the living cells of the germinativum to the zone of the dead Stratum corneum, also called as horny layer which consists of compacted, dead, keratinized cells with a density of 1.55. Because of the dense nature of stratum corneum, values of diffusion coefficient in this tissue are very much smaller than any other skin tissue, which results in higher resistance and greater impenetrability. Hence, stratum corneum is responsible for the barrier function of the skin.

2. Dermis: The dermis (or corneum) essentially consists of 80 % of protein in the matrix of mucopolysaccharides (ground substance). Protein including collagen and elastin fibers are oriented parallel to the epidermis. The dermis supports and interacts with the epidermis facilitating its conformation to the underlying muscles and bones. Blood vessels, lymphatic, nerves as well as the epidermal appendages such as the hair follicles, sebaceous glands and sweat glands are mainly contained and supported within the dermis. Beneath the dermis, the fibrous tissue opens out and merges with the fat-containing subcutaneous tissue.

3. Subcutaneous fat layer: Subcutaneous fat layer serves as a cushion for the dermis and epidermis. It also provides a thermal barrier. Collagenous fibers form the dermis thread between the superficial skin layer and subcutaneous layer. Combined, these layers form the skin which is pierced at various places by two types of potential diffusion shunts: hair follicles and sweat glands. These skin appendages however, actually occupy only 0.1 % of the human skin surface.

Skin as a site for transdermal drug administration: The skin is one of the most extensive and readily accessible organs of the human body. The skin of an average adult body covers a surface area of approximately two square meters and receives about one third of the blood circulating through the body. With a thickness of only a few millimeters (2.97 ± 0.28 mm), skin separates the underlying blood circulation network from the outside environment and serves as a barrier against physical and chemical attacks. It acts as a thermostat in maintaining body temperature and shields the body from invasion by microorganisms. Different strategies including the use of skin penetration enhancers, iontophoresis and sonophoresis have been developed to minimize the skin's barrier function.

Transdermal penetration of drugs[3]: Penetration of drugs through skin or percutaneous absorption is defined as the penetration of substances into the various layers of skin and permeation across the skin into systemic circulation. The percutaneous absorption is a stepwise process and can be divided basically into three steps:

i. Penetration: This is the entry of a substance into a particular layer.

ii. Permeation: This is the penetration from one layer into another which is different both functionally and structurally from the first layer.

iii. Absorption: This is the uptake of a substance into the systemic circulation. Diffusion through stratum corneum is purely a passive process.Epidermal diffusion is the first phase and clearance from the dermis is the second, the later being dependent on the effective blood flow

Nanoemulsion: The technological application of nanoemulsions have increasingly been used in various application due to their characteristic properties, small droplet size, high interfacial area ,transparent or translucent appearance, high solubilization capacity, flocculation and in some cases ,the coalescence.[1-3] Nanoemulsions are novel drug delivery system consisting of emulsified oil and water systems with mean droplet diameters ranging from 50 to 1000 nm. Usually the average droplet size is between 100 and 500 nm and can exist as oil-in-water (o/w) or water- in- oil(w/o) form, where the core particle is either oil or water, respectively.

Nanoemulgel[3,4]

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Advantages of Nanoemulgel:

A stable nanoemulsion formulation is enhanced through nanoemulgel, by decreasing surface and interfacial tension and which leads the viscosity of the aqueous phase to be increased.

Emulsifier and thickeners been added to hold the gelling capability of hydrogel serves a better stability, permeation.

The Nanoemulgel formulation is known to support better delivery of lipophilic and poorly soluble drugs.

Disadvantages of Nanoemulgel:

1. Bubbles formed during emulgel formulation.

2. Possibility of allergenic reactions.

Material:

Terbinafine HCl (cipla pharmaceutical pvt ltd, Mumbai); Capryol oil(Gattefose); Peceol oil(Gattefose); Tween 80(Fine chem) Propylene glycol(Fine chem); Polyethylene glycol(Fine chem); Transcutol p(Gattefose); ethanol (Loba chem); Carbapol 940 (Loba chem); Carbapol 934(Loba chem). All other ingredients used were of analytical grade.

Methods[5,6]:

High energy emulsification method:

-The ultrasonic waves is also used to create the cavitation bubble that collapse and locally release a substantial amount of energy into the system which generate smaller droplets of the internal phase with uniform distribution.

-High pressure homogenization offers intense turbulence and shear flow to the oil and water mixture under high pressure. Severe turbulence leads to the breaking of dispersed phase into small droplets.

UV-spectrophotometric method

a. Preparation of standard stock solution

Accurately weighed 10mg of terbinafine Hcl was treansfered to a 100ml volume flask, Dissolved in 20 ml distilled water by shaking manually for 10 min. The volume was adjusted with the same up to the mark to give final strength i.e. 100 µg/ml

b. selection of wavelength for analysis of terbinafine Hcl : appropriate volume 0.5 ml of standard stock solution of terbinafine Hcl was transferred into 10 ml volume flask, diluted to mark with d/w to give concentration of 5 µg/ml. The resulting solution was scanned in the UV range (400-200 nm) in spectrum Terbinafine Hcl showed absorbance maximum at 283 nm.

Characterization of drug:

Description: - white crystaline powder.

Melting point determination:-The melting point of the drug matched with the values found in literature indicating the quality of sample.

Table 1: Melting point of Terbinafine HCl

Drug	**Melting point**	
	Practical	**Standard**
Terbinafine HCl	196°c-197°c	195°c-198°c

Melting point of Terbinafine HCl was found to be in the range of 196-197^{0}C as reported in literature, thus indicating quality of the drug sample.

FTIR of drug:- for functional group detection

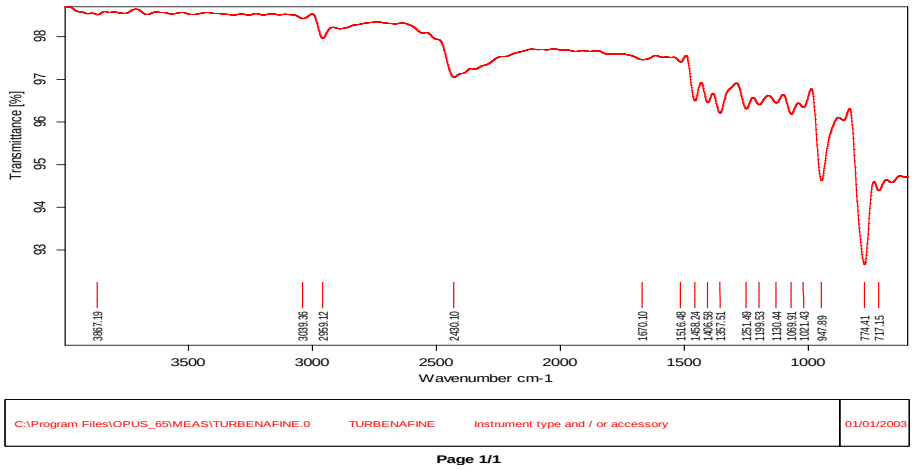

Figure 1 - IR spectra of Terbinafine HCl.

Table 2 - Reported IR frequencies of terbinafine HCl.

Functional Group	**Value cm^{-1}**	**Std range cm^{-1}**
C≡C	2150	2250-2120
C = C	1670	1780-1650
N-H	3650	3700-3500
C-H	3000	3100-3000
C-N	1251	1340-1250

The IR spectrum of the pure Terbinafine HCl sample was recorded by FT-IR spectrometer as shown in which was compared with standard functional group frequencies of Terbinafine HCl

UV-spectrophotometric method

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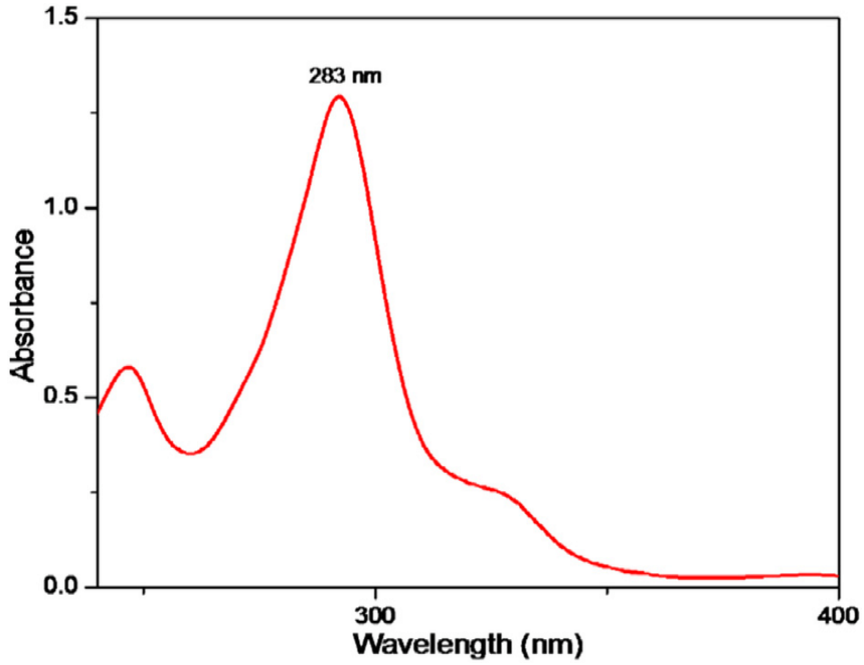


Figure 2: Determination of λ_{max} Terbinafine HCl.

Calibration curve of Terbinafine HCl in phosphate buffer solvent pH 5.5

From the stock solution of Terbinafine HCl known concentration of 100µg/ml is prepared by suitable dilution with pH 5.5 buffer solutions. Wavelength scanned for the maximum absorption of drug solution using UV spectrophotometer within the wavelength region of 200–400 nm against blank phosphate buffer. Obtained spectra shows the peak with a highest absorbance is considered as absorbance maximum of the drug.

Table 3- Absorbance of terbinafine HCl.

Sr.no	Concentration(ppm)	Absorbance
1	5	0.0876
2	10	0.185
3	15	0.265
4	20	0.466
5	25	0.539

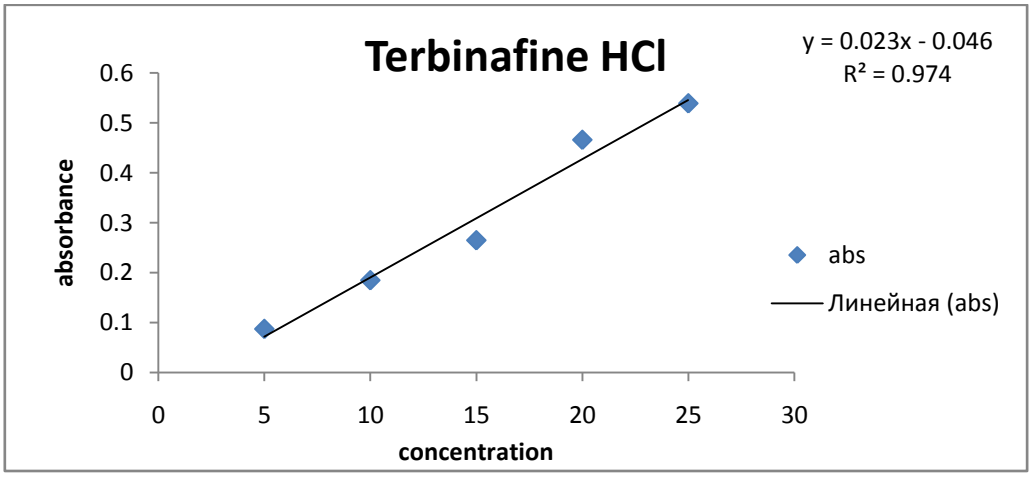


Figure 3: Calibration curve of Terbinafine HCl.

Charecterization of excipients:

Construction of pseudo ternary phase diagram

Development of pseudoternary diagram On the basis of the solubility study pseudoternary phase diagrams were developed in order to obtain the nanoemulsion zone and determine the concentration ratios of the components of nanoemulsion formulae.

Thermodynamic stability studies

The prepared nanoemulsion formulae were subjected to different thermodynamic stability tests . To overcome the problem of metastable formation, thermodynamic stability tests were performed. The selected formulae were subjected to different stress tests, such as centrifugation, heating cooling cycle, and freeze-thaw cycle tests. The formulae that survived thermodynamic stability tests were subjected to further characterization, such as droplet size, viscosity determination. All the tested formulae passed the tests, i.e. there was no sign of phase separation, turbidity or drug precipitation observed.

Preparation Methods: for preparation of terbinafine Hcl nanoemulgel

A) Preparation of nano Emulsions:

The formulations were developed by varying the proportions of Smix –tween 80 & propylene glycol(45%-50%), capryol oil (10%-15%), and water(45-50%),that selected according to pseudo ternary phase diagram study. Initially, a pre-emulsion was prepared by homogenous mixing of the three components in a magnetic stirrer (500 rpm, 70◦C).

The pre-emulsion was subsequently homogenized using a probe sonicator (6 mm) probe operating at a ultrasonic frequency 50 ampitude. The sonication was carried out for 20 cycles (6 s on time and 2 s off). The homogenization was carried out in an ice-bath to compensate the heat generated.

- **Selection of concentration for nanoemulsion:**

The composition of nanoemulsion, was established in our earlier study which consist of capryol oil, tween 80, propylene glycol, dissolve terbinafine Hcl 1% in above respected solution, on magnetic stirrer. That mixture are kept on probe sonicator for size reduction purpose. Different trial batches are taken for selection of appropriate S_{mix} ratio, surfactant concentration, that batches taken that are as following:

Table 4: trial batches of nanoemulsion

Trial Batch no	**Drug(%)**	**S_{mix}**	**Oil : S_{mix}**
1.	1	1:1	1:6
2.	1	1:1	1:4
3.	1	1:2	1:6
4.	1	1:2	1:4
5.	1	1:3	1:6
6.	1	1:3	1:4

(B) Preparation of gel:

Add 1-5% of carbapol $_{940}$ in appropriate quantity of distilled water with continuous stirring, to get uniform spreadable gel.

Selection of concentration for emulgel: Nanoemulgel and carbapol 940 gel are taken in different proportion that are as following: Nano emulsion was mixed with the gel in 1:1 ratio with gentle stirring to obtain nanoemulgel. pH adjuster used according to requirement of pH.

Table 5: Trial batches of nano emulgel.

Trial batch no.	Nanoemulsion : carbapol 940
1.	1 : 0.5
2.	1 : 1
3.	1 : 1.5
4.	1 : 2
5.	1 : 2.5

Characterization of optimized TB nanoemulsion formulae

Measurement of droplet size: The average droplet size and polydispersity index (PDI) of the nanoemulsion formulae were measured by Dynamic Light Scattering technique (DLS) or sometimes called as photon correlation spectroscopy (PCS) using a Malvern Zetasizer Nano ZS90, UK. The measurements were performed at a fixed angle of 90° and at 25°C. About 0.1 mL of drug loaded nanoemulsion was dispersed in 10 mL of distilled water under gentle stirring in a glass beaker .Then sample cell was filled with the dispersion for droplet size measurement. Each size value was the mean of triplicate samples±SD. The PDIwas measured for the uniformity of particles diameter.

Zeta potential measurement

Zeta potential of TB nanoemulsions was measured by an electrophoretic light scattering technique using a Malvern Zetasizer Nano ZS (Malvern Instruments, Ltd., UK) with a dynamic light-scattering particle-size analyzer at a wavelength of 633 nm by applying 1v electric field. Nanoemulsions were dispersed in prefiltered, double distilled water at ratio 1:100. The average ± SD of three independent measurements were reported.

Determination of pH: The pH values of TB nanoemulsion formulae were determined using a digital pH meter, the measurements of pH data was done in triplicate.

Viscosity determination

Brookfield DV I viscometer (Brookfield Engineering Laboratories) using Spindle 2 # at 25 ± 0.5 °C the spindle speed began at 50 rpm.

Refractive Index: Refractive Index of formulae was measured to evaluate the nanoemulsion transparency at 25 °C using Refractometer Abbe,

In vitro drug diffusion study:

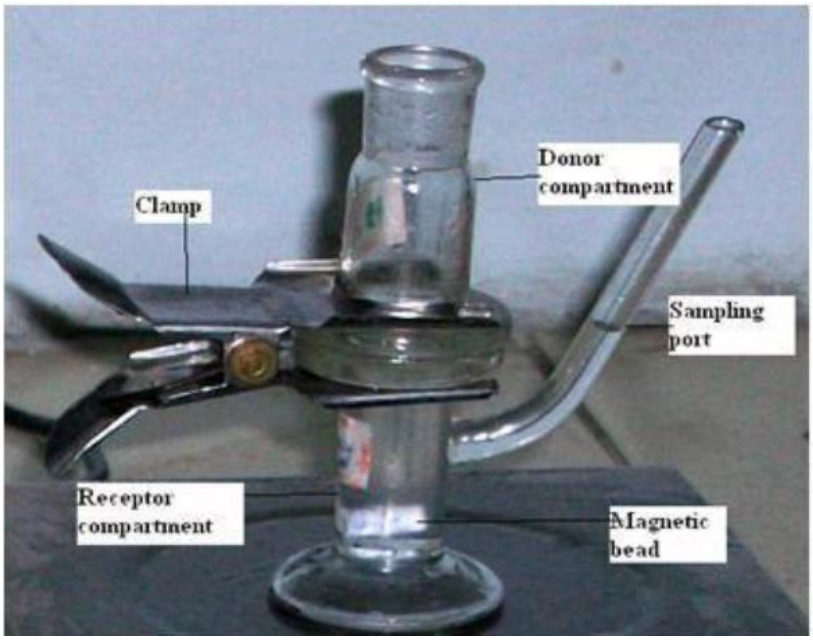


Figure 4: diffusion study apparatus.

Drug release from optimized nanoemulsion formulae was assessed using the dialysis bag method through semipermeable membrane. The dialysis bag was fixed to a 500mL stoppered glass container containing 350 mL of the release medium pH 5.5 (methanol 10%/acetate buffer 1:9, respectively).

The optimized nanoemulsion formulae (1 mL), equivalent to 10 mg TB, were separately added into the dialysis bag. Samples were withdrawn at fixed time intervals (0.5, 1, 2, 3, 4, 5 and 6 h) and a corresponding aliquot of the fresh dissolution medium was replaced. The samples were analyzed spectrophotometrically at λ max 283 nm. All experiments were performed in duplicate.

Preparation of Terbinafine nanoemulsion based gel:

Optimized TB loaded nanoemulsion formulae were incorporated with the polymer gel (1 %) matrix separately in a ratio 1:1 forming viscous and smooth nanoemulsion based emulgel (F1-F3, respectively) using a magnetic stirrer for 15 min at 250 rpm.

Drug content: The drug content of the different preparations TB nanoemulsion based gel was determined separately by dissolving 0.1 g of the gel, containing 0.5 mg of the drug in 25 mL of methanol. The resulting solutions were filtered with whatmann filter paper to obtain clear solutions. The drug content was measured spectrophotometrically at l max 283 nm against methanol as blank.

Results and discussion [3,5,6,7,8]

Solubility study

Solubility of TB was determined in various oils, surfactants and co-solvents mixtures (Fig. 1). Solubilizing capacity of an oily phase is critical for development of nanoemulsion as it determines drug loading efficacy. Oily phase screened include; peceol oil, caproyl 90 and peceol oil. Among all the screened oils high solubilization capacity was exhibited in capryol 90 oil, followed by pecol oil with the lowest solubility. Therefore, capryol oil was selected for further investigation. This difference may be due to the relative hydrophobicity of the oils as capryol 90 ® oil has the HLB value; 6 compared to HLB values of other oil. Non-ionic surfactants are usually utilized for fabrication of nanoemulsion as they are less toxic compared to the ionic counterparts Another important criteria in surfactants' selection is bioactivity aspect. Some surfactants are recognized as safe (GRAS) bioactive excipients due to hydrophilic character as Tween 80 or inhibitory effect on P-glycoprotein . In this Tween 80 has the highest solubility capacity of TB (27.4 mg/mL±1.88) and it is an HLB value of 14.5, which was suitable for the preparation of oil in water NE. Different co-solvents namely; Propylene glycol,were assessed in this study for the solubilization ability of TB, that demonstrated that the highest solubility for TB was in propylene glycol. The solubility of all excipients is carried out in different solvent and summarized in below**: -**

Table no 6: Solubility study of excipient.

Excipient	**Solubility**	**Standard**
Capryol oil	Slightly soluble in water and soluble in oils and organic solvent.	Miscible with ethanol, methanol and insoluble in water
Tween 80	Soluble in water and ethanol, methanol.	Soluble in water and organic solvent.
Propylene glycol	Miscible with methanol, propyl alcohol, chloroform, ether, glycerin, water.	Alcohol is miscible with chloroform, ether, glycerin, and water.
Carbopol 940	Soluble in water	Soluble in water,

From the solubility of all excipients it can be concluded that all excipients are same as standard specified in the handbook of pharmaceutical excipients.

Solubility analysis of drug in different excipients:- One important consideration when formulating a nanoemulsion drug delivery formulation is to avoid precipitation of the drug in vivo a solubility of Terbinafine HCL in different oils, surfactant and co-surfactant is shown in following figure:

a. Solubility in oil:-

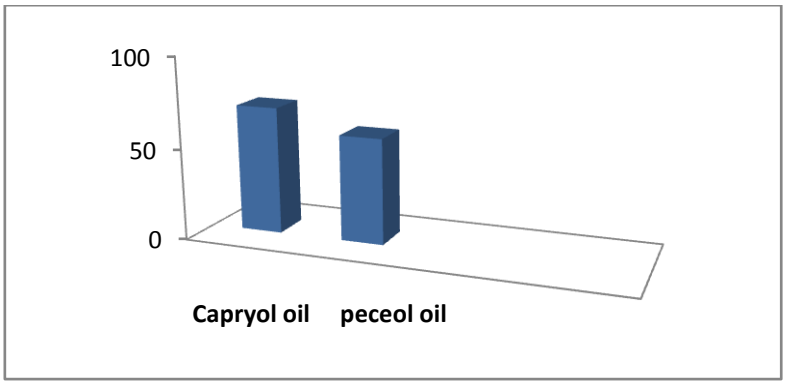


Figure 5: Solubility of Drug in different oils.

b. Solubility in Co- Surfactant:-

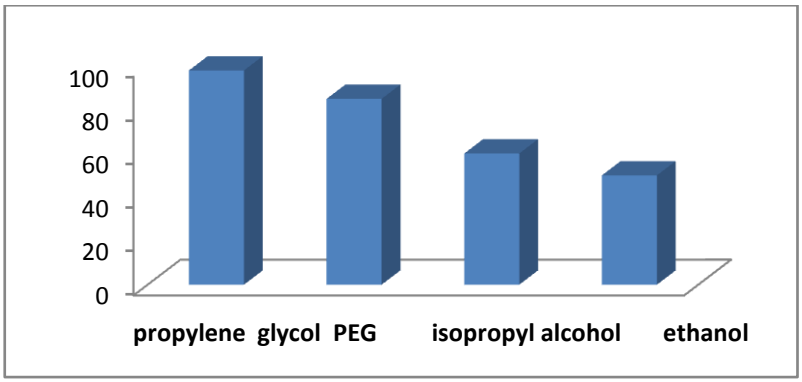


Figure 6: Solubility of Drug in different Co-surfactants.

c. Solubility in Surfactant:-

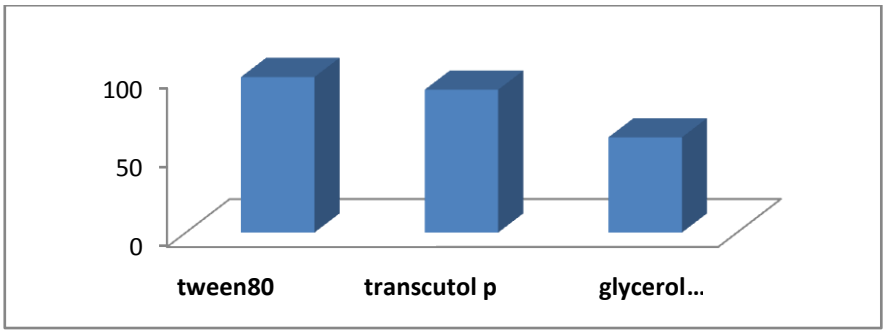


Figure 7: Solubility of Drug In Different Surfactant.

Terbinafine HCl exhibit good solubility in capryol oil out of 2 oils. Terbinafine HCl shows the higher solubility in out of 3 surfactant Tween 80 .In case of co-surfactant Propylene glycol shows good solubility , all 3 component selected for further study.

The solubility of TB was determined in various excepients; oils

Caproyl 90(52 mg/ml), Peceol(46 mg/ml), surfactants Tween80(25mg/ml) and co-solvents Transcutol (80mg/ml), Propylene glycol (92mg/ml). An excess quantity of TB (0.1 gm) was mixed with 2 mL solvent (oils, surfactants and co-solvents) separately in stoppered vials for 14-15 hr at 37^0 C and 200-300 rpm in magnetic stirrer. The solvent-drug mixture was then shaking for 1hr. The supernatant was filtered through wattman filter paper, diluted with methanol. The amount of TB solubilized was quantified spectrophotometrically at λmax 283 nm.

Development of pseudoternary diagram

Pseudoternary phase diagrams were constructed in the absence of TB to recognize the zone of nanoemulsion and to optimize the concentration of oil, surfactant and co-solvent in the nanoemulsion formulae. The ratio of surfactant to co-solvent was very effective for stable and efficient nanoemulsion formation. The phase diagrams were constructed at the ratio of surfactant/co-solvent 1:1, 1:2, 1:3. The diagrams showed the biggest nanoemulsion areas are; 1:1, 1:2, 1:3, 40 .0 % ,45.09%, 50.06%, respectively compared to areas in case of surfactant/cosolvent 2:1, 3:1, 4:1. The larger the area of the nanoemulsion field, the greater the nanoemulsification efficiency of system . It was noticed that the increase of the co-solvent concentration with respect to surfactant caused corresponding increase in the nanoemulsion area.

Evaluation of TB-loaded nanoemulsions

Organoleptic characterization:-

Table-7: Different type of organoleptic characterization of all excipients.

Excipients	**observation**	**Standard**
Capryol oil	Was Found to be colourless and clear transparent oil	Colourless and clear transparent oil
Tween 80	Was Found to be yellow and clear transparent oil	Yellow and clear transparent oil
Propylene glycol	Was Found to be colourless and clear transparent liquid	Colourless and clear transparent liquid
Carbapol 940	Was found to be white powder	White powder

From the organoleptic characterization of all excipients it can be concluded that all excipients are same as standard specified in the handbook of pharmaceutical excipients.

Thermodynamic stability tests:

Heating and cooling cycle: The nanoemulsion formulae were subjected to 3 cooling, heating cycles between 4^0 C and 45^0 C by storing at each temperature NLT 24 hr. Samples were then observed for precipitation or separation.(6 no. batch selected as optimized batch)

Centrifugation study

The formulations D1-D6 were centrifuged using laboratory centrifuge at 3500 rpm for 30 min. The resultant formulations were then checked for any instability problem, such as phase separation, creaming or cracking. Formulation which is stable selected for further studies.

Table 8: Results of physicochemical character and pH, viscosity and drug content studies of nanoemulsion and nano-emulgel:

Batches	Color	Refractive index	pH	Spreadability		Drug content	Viscosity(cp)	PI	Particle size (nm)	Zeta potential (mV)
				Spread diameter (φ) mm	Spreading area (S)					
1	white	1.335	5.5	65	3295.5	97.3	26.857	0.309	546	-10.04
2	white	1.331	5.6	61	2902.38	98.5	25.978	0.315	452	-15.06
3	white	1.329	5.5	56	2446.08	97.5	26.359	0.323	459	-18.07
4	white	1.332	5.7	70	3822	98.4	25.985	0.318	523	-14.03
5	white	1.331	5.6	90	6318	98.2	27.153	0.318	456	-16.09
6	white	1.334	5.7	93	6425	98.7	26.543	0.326	438	-15.04

Measurement of droplet size and polydispersity index

The droplet size of optimized formulae. The average droplet size of the investigated formulae ranged from nm, which indicated that emulsion droplets are in nanometric range It was observed that systems containing 10% capryol oil produced nanoemulsion with a larger particle size than systems containing 10% capryol i.e. the larger the oil percentage, the larger was the mean droplet size. These findings were consistent with a

previous report showing that the mean droplet size increased significantly when more oil is incorporated owing to the expansion of oil droplets of the nanoemulsion by the further oil addition and due to the simultaneous decrease in the Smix proportion. The surfactant used in the preparation of these nanoemulsion systems could be related to the penetration of co-solvent molecules into the surfactant film.

Zeta potential measurement

Zeta potential (zp) is a function of the surface charge whose value can be related to the physical stability of colloidal dispersions. High absolute zeta potential values (±30 mV) should preferably be achieved in most of the emulsions prepared in order to ensure the creation of a high-energy barrier against coalescence of the dispersed droplets.

Refractive index and pH measurements

Refractive index (RI) is taken as a measure for nanoemulsion transparency. The values of RI of selected TB-loaded nanoemulsion formulae were similar to that of water (1.334), indicating clear and transparent formulae and the prepared nanoemulsions were of o/w type.

Viscosity determination

Low viscosity is one of the characteristic features of the nanoemulsions, which exhibit Newtonian flow behaviour. The study revealed that the viscosity has a tendency to increase with an increase in the oil content. As the oil content was increased from 5% w/w to 10% w/w, an increase in the viscosity of the formulae was observed which might be due to higher oil content. Moreover, the viscosity determination showed that as the concentration of co-solvent increased, the viscosity of the TB-loaded nanoemulsion formulae also gets decreased. This could be related to increased penetration of co-solvent molecules in the surfactant film decreasing the surface viscosity of the interfacial film, and forming transparent systems.

Characterization of Terbinafine nanoemulsion based emulgel

Physicochemical properties and drug content The pH values of all prepared emulgel formulae were 5.715 ± 0.26 which is within the physiological range and considered acceptable to avoid the risk of irritation upon application to the skin adult skin PH is 5.5. Drug contents of the optimized nanoemulsion based gel formulae were found to be 97–98 %, respectively. Since all the nanoemulgel formulae and marketed product carried equal drug load, it could, therefore, be concluded that the concentration gradient is not the factor governing the permeation process. Moreover, hydration of the stratum corneum, due to the external water

phase of the NE, causes the corneum cells to swell, thus making the channels for drug passage wider, resulting in high diffusivity of the lipophilic drug as the droplet size approaches to molecular dispersion. As some lipid chains in the stratum corneum are covalently attached to the corneocytes, hydration of these proteins will also lead to the disorder of the lipid bilayers This might be due to a decreased thermodynamic activity of the drug in the nanoemulsion at the higher content of surfactant.

Determination of pH

The pH of the gels was measured using a digital pH meter (HANNA). The results are the mean of three readings. Range of pH found in between 5.5 to 6.5.

Spreadability:

Spreadability was determined using an apparatus which was adapted in-house. It consists of a wooden block, which was provided by a pulley at one end. By this method, spreadability was measured on the basis of "slip" and "drag" characteristics of the gels. A ground glass slide was fixed on this block. An excess of gel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with a hook. A weight of 100 g was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to a pull of 20 g weight with the help of a string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm was noted. A shorter interval indicates better spreadability. Spreadability (S) was calculated as in Eq 1.

$S = M.L/t$ …… (Eq 1)

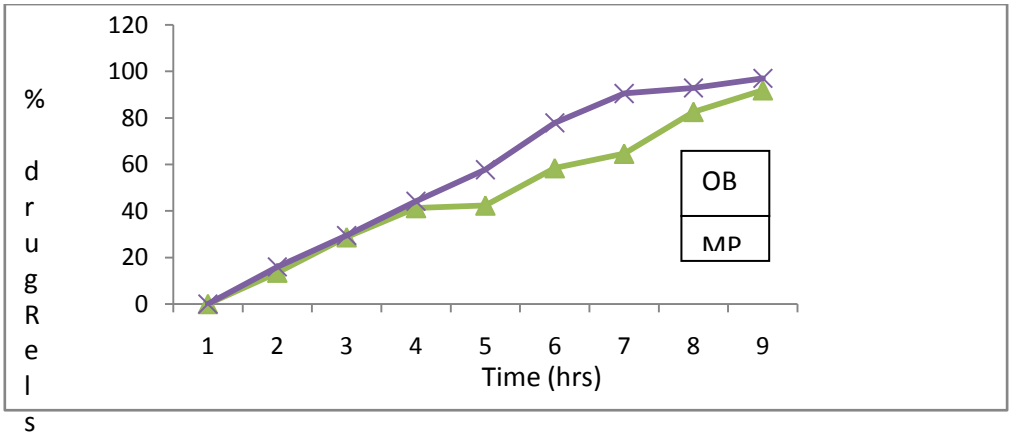
Where M is the weight (g) tied to the upper glass slide, L is the length (cm) moved on the glass slide, and t is time (sec). Determinations were made in triplicates

Drug Content determination: The readings were taken for average of 3 times. Results are shown in table 0.10gm of developed gel was taken and dissolved in 100ml of phosphate buffer of pH 7.0. The volumetric flask containing gel solution was shaken for 2hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered using Millipore filter (0.45µm). After suitable dilution drug absorbance was recorded by using UV- visible spectrophotometer (UV – 1700, Shimadzu, Japan) at λ max

In-vitro Drug Diffusion Study: Cellulose membrane (0.45µm, obtained from sigma chemicals) was used for this study. A sample of 1g of the preparation was spreaded on a cellulose membrane previously soaked overnight in the release medium. The loaded membrane was firmly stretched over the edge of a glass tube of 2 cm diameter; the membrane was tied up with a rubber to prevent leakage .Tubes were then immersed in the dissolution vessel which contained 50 ml of the release medium, phosphate buffer pH 5.5, and maintained at 37ºC ± 0.5ºC25. The shafts were rotated at 50 rpm and aliquots each of 3 ml were withdrawn from the release medium at specified time intervals. Withdrawn samples were replaced by equal volumes of fresh release medium. The samples were assayed spectrophotometrically at λmax**.** In vitro Drug release of the nanoemulgel optimized batch formulation and marketed formulation was studied amongst, optimized batch formulation showed drug release rate same as marketed formulation.

Table 9: In-vitro drug release of nano-emulgel optimized batch formulations and marketed formulation:

Time(Hours)	Optimized batch (drug release in %)	Marketed product (drug release in %)
1	25.6	27.9
2	29	29.9
3	37	38.3
4	43	44.7
5	50.2	49.3
6	60	54
7	72.8	75.5
8	92.2	94.9



Where, OB- Optimized batch, MP- Marketed Product.

Figure 8: Drug Release Profile of Optimized nanoemulgel formulation.

Optimized batch show the same drug release as nearby their marketed formulation.

Discussion

Terbinafine HCl is a Terbinafine Hcl (TB) is a synthetic allyl amine derivative with antifungal activity. TB exerts its effect through inhibition of squalene epoxidase, thereby blocking the biosynthesis of ergosterol, an important component of fungal cell membranes. As a result, this agent disrupts the fungal cell membrane synthesis and inhibits the fungal growth. as jock itch (tinea cruris), athlete's foot (tinea pedis), and other types of ringworm (tinea corporis) and Candida species. The objectives of the present study are improving solubility and antifungal activity of TB through incorporation in nanoemulsion based gel .TB is slightly soluble in water.

As part of Preformulation studies, solubility of drug in various oils, surfactant and co-surfactant was determined to enable selection of excipients with good drug loading ability. Selected oil (Capryol 90 oil), surfactants (tween 80), and co –surfactants (propylene glycol). Surfactant and co-surfactant was tried at 1:3 ratio. Formulation were optimised on two test *i.e.*centrifugation test, heating and cooling cycle test. Formulation F these six formulations selected for from various Smix (1:1,1:2,1:3,2:3) ratios which passed Centrifugation Test, Heating and cooling cycle, *i.e.* these four formulation were optimized formulation. Optimized formulations were further characterized for percent transmittance.

Among all these formulation the D6. formulation is optimized formulation because it shows good percent transmittance . In case of in vitro diffusion test final formulation showed 87.2% drug release within 8 hours .

Optimized naoemulsions were converted into nanoemulgel to overcome the problem associated with topical application of nanoemulsion. Formulation D3, D4, D5, D6, these Four formulations selected from various ratio of Carbopol 940 (gelling agent) (1 : 1) . Optimized formulations were further characterized for pH, viscosity, spreadability, drug content determination. Among all these formulation the E3 formulation is optimized formulation because it shows good drug content. In vitro diffusion test for E3 formulation showed 94.3% drug release within 8 hours .

The nanoemulgel developed for Terbinafine HCl can offers many advantages over conventional dosage form including dose adjustment according patient requirement, reduction in dose related toxicity, related to drug related toxicity, cost effective manufacturing.

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Author Contributions:

Ms.Kamal Sadaphal[*] contributed for overall work design, result in interpretation and manuscript preparation.

Dr. Kishor Salunkhe contributed for guidance to prepare nanoparticle for formation nanoemulgel.

Dr. Machhindra Chavhan, contributed for provide the all necessary requirement, chemical in laboratory.

Ms.Utkarsha Lasure contributed for spectral charecterisation, interpretation of results and manuscript preparation.

Conflict of Interests

The authors declare that they have no conflict of interest.

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