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Research Article

FORMULATION AND EVALUATION OF THERMOSENSITIVE HYDROGEL OF TERBINAFINE HYDROCHLORIDE FOR TOPICAL DRUG DELIVERY

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A B S T R A C T

Stimuli-sensitive hydrogels change their swelling behavior and drug release by sensing changes in the surrounding environment. One example is temperature-sensitive hydrogels which change their swelling behavior in response to a change in the environmental temperature. Poloxamers are tri-block copolymers that exhibit thermoreversibleproperties by transforming from a liquid-like behavior to gel-like behavior above acertain temperature called sol-gel transition temperature. By varying the concentration of poloxamer and other excipients, hydrogels with sol-gel transition point close to bodytemperature can be achieved. The aim of the present study was to develop poloxamerhydrogels as *in situ* gelling formulation for topical drug delivery. The anti-fungal drug Terbinafine was used as a model drug substance. First, pre-formulation work on the solubility of Terbinafine in different co-solvents was performed. Then, eight different formulations containing 1% Terbinafine in poloxamer and a particular co-solvent (propyleneivglycol or Transcutol®-P) of various concentrations were prepared. The formulations were characterized for transition temperatures, rheological, mechanical, and mucoadhesiveproperties.

Terbinafine permeability and antifungal effect of the systems were evaluated. Except for one formulation, all hydrogels exhibited thermo sensitive property, i.e. changing from Newtonian (liquid-like) behavior at 20°C to non-Newtonian (gel-like) behavior at 37°C. Transcutol increased the transition temperature of the formulations, while the opposite effect was observed for propylene glycol. At body temperature, formulations with high poloxamer concentrations (17%) rendered gels with higher valuesin viscosity, compressibility and hardness. Formulations containing 17% poloxamer and20% Transcutol-P and 10% propylene glycol, respectively, exhibited high values in both adhesiveness and work of adhesion. No significant differences in the permeability and antifungal activity of Terbinafine were observed between the formulations. The latter suggests no influence of the gel vehicles on the biological effect of Terbinafine. Based on the results, formulations containing 17% poloxamer and 20% Transcutol-P and 10%propylene glycol, respectively, seemed to be promising thermosensitive systems for topical drug delivery.

INTRODUCTION

A **topical delivery system** is one that is applied directly to an external body surface either by spraying, or dusting it on or by instilling it. The products that are applied on easily accessible mucosal membranes (ocular, nasal, oral, vaginal and anal mucosal membrane) are also considered as topical drug delivery systems. The aim of topical drug delivery is to get a local action only, in contrast to other dosage forms such as parenteral and oral dosage form which are meant for systemic action.[1]

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Hydrogels

Hydrogel is a transparent, viscoelastic and thermo dynamically stable system consisting of a polar solvent and a polymer, where polar solvent is the external phase. The polymer which are of synthetic or natural origin assemble to form a three dimensional network which can absorb and retain significant amount of water.[2]

Hydrogels are one of the upcoming classes of polymer-based controlled release drug delivery systems.[3,4] Polymeric drug delivery systems have been extensively studied in order to solve the potential problems associated with drugs or bioactive molecules including toxicity, site dependence, low effectiveness, poor solubility, short half-life, rapid degeneration and rapid clearance from the body. Considering various properties such as flexibility, structure,

biocompatibility, and hydrophilicity, three dimensional matrices, hydrogels, are being extensively used as drug delivery carriers. Hydrogels are three dimensional structures of hydrophobic polymer network consisting of a single chain of monomers being cross-linked or chains of co-polymers being cross linked.[3]These two are insoluble in water due to ionic interaction and hydrogen boding between the crosslinking, but they become swollen by imbibing large amount of biological fluids or water.[4] Even then, hydrogels maintain their physical integrity and mechanical strength due to their cross-linking.

Hydrogels generally posses a good bio compatibility because of their hydrophilic surface, which has a low interfacial free energy. Also, the soft and rubbery nature of hydrogels minimizes irritation to surroundings tissue increasing bio compatibility.[5]

Hydrogels can also be classified into conventional and stimuli-sensitive hydrogels.

Stimuli-sensitive hydrogels

Stimuli-sensitive hydrogels can change their swelling behavior by sensing changes in surrounding environment. The environmental changes or stimuli can be in the form of 6 temperature, pH, light, ionic strength, glucose, pressure, magnetic field or ultra sound.

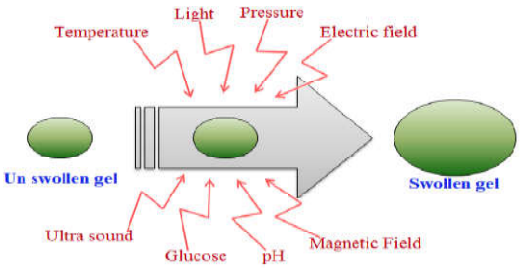

Figure 1 Schematic representation of different stimuli on polymer matrix

Temperature-sensitive poloxamer-based hydrogels

Temperature-sensitive hydrogels are well-accepted form of hydrogels; they show variability in the swelling behavior in response to changes in the environmental 8 temperature.[6] Temperature-sensitive hydrogels undergo phase transition (solid to liquid /liquid to solid or swelling/shrinking of polymer network) with the change in temperature above or below certain temperature called critical solution temperature (CST). Based on mechanism, these hydrogels are divided into positive and negative temperature-sensitive polymers. Most of the polymers belong to positive temperature sensitive hydrogels, which swells in water with increase in temperature above critical point called upper critical solution temperature (UCST). In other words, polymers with UCST generally become gel with increase in temperature and by decreasing temperature below UCST the polymer network shrinks. On other hand, negative hydrogels shrinks with increase in temperature above critical temperature called lower critical solution temperature (LCST).[7] Polymers with LCST releases drug by shrinking the polymer network with increase in temperature. Depending on the type of polymers used, temperature-sensitive hydrogels can be both biodegradable and biocompatible. Various drug classes, such as hormones, anti-diabetic agents, anticancer agents and protein and peptides, have been investigated for enhanced delivery by the use of such systems.[3]

The focus of the present thesis will be on poloxamers in the preparation and characterization of thermosensitive hydrogels. 9 Poloxamer block co-polymers are introduced in 1950s are being used in large areas of liquids, pastes and solid. They are now listed in US and European pharmacopeias.[8] Poloxamers show reversible temperature-sensitive properties, which present great interest in optimizing drug formulation. Poloxamers are odorless and tasteless, waxy white granules of free-flowing nature. They are generally soluble in any solvent (organic/ aqueous /polar/non-polar). The aqueous solutions of poloxamer are very stable in the presence of alkali, acid or metal ions. Due to these properties poloxamers established themselves as preferred substance in formulation techniques.[9] The poloxamers are marketed in various grades, which have different physical and chemical properties.

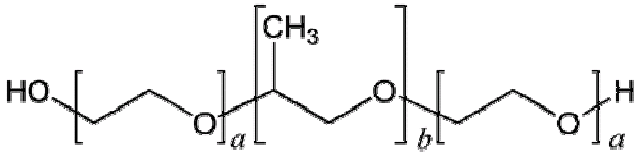

Figure 2 General structure of poloxamers

Poloxamer 407 is a commonly used co-polymer in pharmaceutical formulations. Poloxamer 407 has a HLB of 22 at 22°C and is also presented as an "inactive" ingredient by FDA in different types of formulations.[10,11] Aqueous solutions of poloxamer 407 show thermoreversible properties; i.e. they transform forth and back, from a liquid-like behavior to gel-like behavior above a certain temperature called sol-gel transition temperature or critical transition temperature. Above sol-gel transition temperature the solutions behave as solid and below, it behaves as a liquid. The gelling process of poloxamer is typically divided into two steps as shown in figure 1. The first step occurs when the temperature is increased reaching the critical micelle temperature and the poloxamer co-polymers aggregate to form spherical micelles.

These micelles consist of an outer shell of hydrated swollen poly EO chains with dehydrated poly PO blocks as the core. The process develops into the second step when a further increase in the temperature packs the micelles in an orderly manner to form gels.

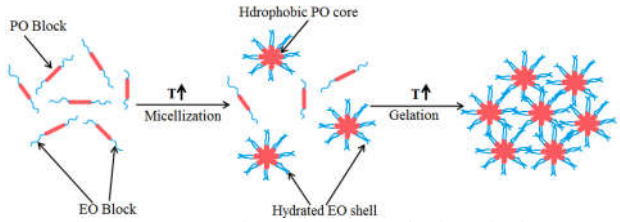

Figure 2 Schematic representation of gelation mechanism of poloxamer 407 in water

Apart from temperature, gelation is also dependent on the concentration of poloxamer molecules in solution.[12]The formation of gels occurs when the concentration of poloxamer is above critical micellar concentration. Poloxamer solution of 20-30% concentrations forms clear liquid at cold temperature 4-5 °C and gel at room temperature. The gel can return to liquid by cooling. Poloxamer formulations are prepared mainly using cold method. In the cold method, the poloxamer polymer is added to cooled water 4-5°C and stirred until homogenous solution is formed. When poloxamers placed in cold water, hydration layer surrounds the poloxamer molecule. The

hydrophobic portions of poloxamer are separated due to hydrogen bonding between water and hydrophilic chains.

The hydrogen bonds break by increase in the temperature, resulting hydrophobic interactions among poly PO chains, thus forming gel.

MATERIAL AND METHOD

Terbinafine hydrochloride received as gift sample from Windlas Biotech, Dehradun. Polaxomer was received as gift sample from Salvus Pharma, Chandigarh. Transcutol-p was received as gift sample from Windlas Biotech, Dehradun. Propylene glycol received from Schelude pharma, Roorkee. Other chemicals are purchased from manoj distributor from roorkeee like Methanol (changshu hongs heng fine chemical), Ethanol (Fisher scientific), HCL, Sodiumphosphatedibasic and Sodiumphosphatemonobasic (Pioneer).

Preformulation Studies

Drug analysis (Identification of drug)

Determination of melting point

Capillary fusion method was used to determine the melting point of Terbinafine Hydrochloride using capillary melting point apparatus. The melting point was recorded and compared to literature value and reported in Table 1.

Table 1 Melting point analysis data

Method used	Experimental value	Literature value
Capillary fusion method	204˚C±0.14	204-208˚C

Determination of absorption maxima (λ_{max}) for analysis

Solutions of Terbinafine hydrochloride were prepared in the different dissolution medium and organic solvents. λ_{max} was determined by scanning between 200-400 nm, using Shimadzu spectrophotometer. The scanned λ_{max} values were compared with literature value. λ_{max} values are shown in Table 2.

Table 2 λ_{max} of Terbinafine Hydrochloride in different dissolution medium and organic solvents

S.No	Solvents	λ_{max} (nm)
1	Methanol	282
2	Phosphate buffer (pH 7.4)	282

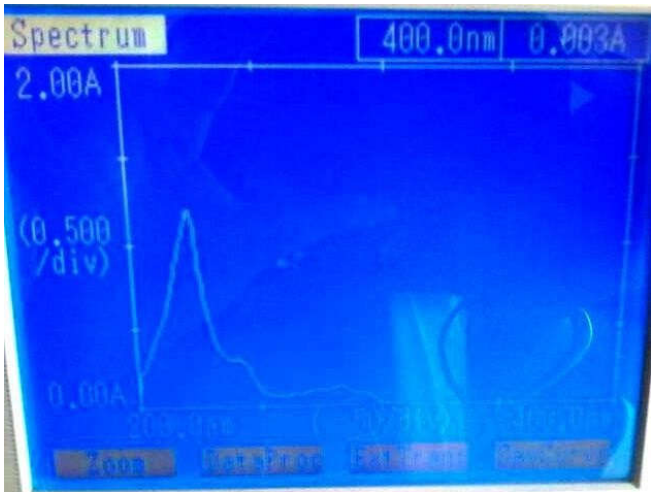

Fig 3 Scanned Graph of Terbinafine Hydrochloride

Fourier Transform Infra Red Analysis

The FTIR analysis is the most powerful technique for qualitative compound identification. The main application of FTIR spectrophotometry is determination of the identity of a compound by means of spectral comparison with that of an authentic sample and verification of the presence of functional groups in an unknown molecule. I.R spectra of pure terbinafine hydrochloride is shown in Fig 4. Pure terbinafine hydrochloride shows sharp characteristics peaks at 2964.56 cm^{-1} for CH stretching, 2431.63 cm^{-1} for C≡C stretching, 1513.22 cm^{-1} for C=C stretching, 1410.02 for alkane C-H bending and 1255.46 cm^{-1} for C-N stretching.

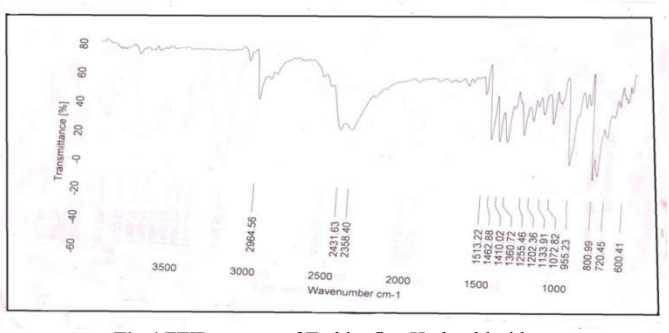

Fig 4 FTIR spectra of Terbinafine Hydrochloride

Calibration curve

Calibration curve of Terbinafine hydrochloride in phosphate buffer pH 7.4

- **Preparation of primary stock solution:** 10 mg of Terbinafine Hydrochloride was accurately weighed and dissolved in small quantity of phosphate buffer pH 7.4 in 10 ml of volumetric flask and volume was made up to 10 ml with phosphate buffer pH 7.4 to produce stock solution having a concentration of 1000 µg/ml.
- **Preparation of secondary stock solution:** From the primary stock solution, 1 ml of solution was taken in the 10 ml of volumetric flask and diluted up to 10 ml with phosphate buffer pH 7.4 to produce secondary stock solution having concentration of 100 µg/ml.
- **Preparation of aliquots:** Aliquots having concentration range of 2-20 µg/ml was prepared by approximately diluting the secondary stock solution with phosphate buffer pH 7.4 separately. The absorbance ofeach aliquot was measured at λ max 282 nm using phosphate buffer pH 7.4 as a blank respectively & standard curve was plotted between concentration in µg/ml on X-axis & absorbance on Y-axis.

Table 3 Calibration Data of Terbinafine hydrochloride in Phosphate Buffer 7.4

S. No	Concentration (µg/ml)	Absorbance
0	0	0
1	2	0.011±0.002
2	4	0.019±0.006
3	6	0.028±0.009
4	8	0.037±0.005
5	10	0.045±0.001
6	12	0.054±0.007
7	14	0.066±0.002
8	16	0.073±0.006
9	18	0.082±0.002
10	20	0.094±0.004

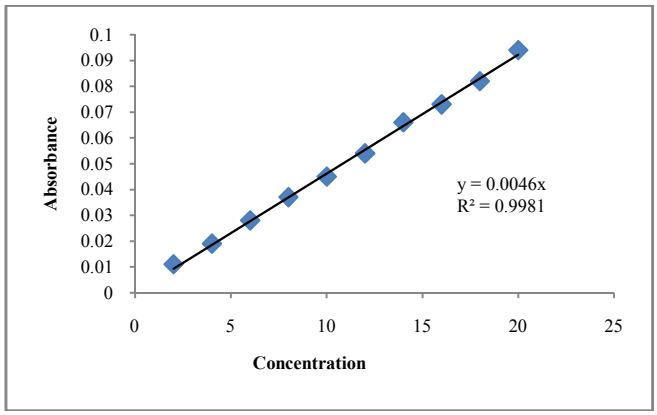

Fig 5 Calibration Curve of Terbinafine Hydrochloride in Phosphate Buffer pH 7.4

Calibration curve of Terbinafine hydrochloride in methanol

- **Preparation of primary stock solution:** 10 mg of Terbinafine Hydrochloride was accurately weighed and dissolved in small quantity of methanol in 10 ml of volumetric flask and volume was made up to 10 ml with methanol to produce stock solution having a concentration of 1000 µg/ml.
- **Preparation of secondary stock solution:** From the primary stock solution, 1 ml of solution was taken in the 10 ml of volumetric flask and diluted up to 10 ml with methanol to produce secondary stock solution having concentration of 100 µg/ml.
- **Preparation of aliquots:** Aliquots having concentration range of 2-20 µg/ml was prepared by approximately diluting the secondary stock solution with methanol separately. The absorbance ofeach aliquot was measured at λ max 282 nm using methanol as a blank respectively & standard curve was plotted between concentration in µg/ml on X-axis & absorbance on Y-axis.

Table 4 Calibration Data of Terbinafine hydrochloride in Methanol

S. No	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.093±0.006
3	4	0.193±0.002
4	6	0.269±0.004
5	8	0.340±0.002
6	10	0.384±0.002
7	12	0.488±0.006
8	14	0.560±0.008
9	16	0.656±0.006
10	18	0.741±0.004
11	20	0.833±0.002

±SD n=3

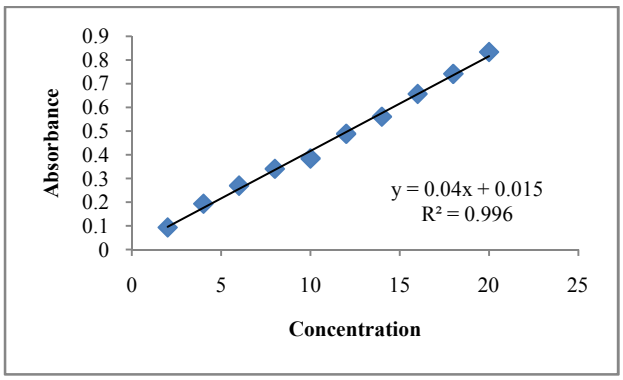

Fig. 6 Calibration Curve of Terbinafine Hydrochloride in Methanol

Solubility study

Qualitative solubility of Terbinafine Hydrochloride in different solvents

The solubility was carried out in different solvents like methanol, ethanol, hydrochloric acid and water. A pinch of Terbinafine Hydrochloride was added into separate test tubes, containing 5 ml of each solvent. The entire test tubes were shaken for 5- 10 min. Then the solubility was visually determined and following results were obtained.

Table 5 Qualitative Solubility of Terbinafine Hydrochloride in Different Solvents at Room Temperature

S. No	Solvents	Solubility
1	Methanol	+++
2	Ethanol	+++
3	.1N HCL	+
4	Water	+

Quantitative solubility of Terbinafine Hydrochloride

The solubility of Terbinafine Hydrochloride was tested in water, phosphate buffer pH 7.4 and methanol. 10 mg of drug was dissolved in 10 ml of distilled water, phosphate buffer pH 7.4and methanol in 10 ml volumetric flasks. The mouth of flask was properly covered with aluminium foil and placed in water bath shaker maintained at 37°C for 24 h, then shaker was switched off and temperature of water bath was maintained again for 12 h. This was done to avoid forced solubility due to shaking. Samples were taken manually and filtered. The UV absorbance of the solution after appropriate dilutions was determined at 282 nm using UV spectrophotometer (Schimadzu-1700 UV-Visible spectrophotometer) and the amount of drug dissolved was calculated using calibration curve.

Table 6 Quantitative Solubility of Terbinafine Hydrochloride at Room Temperature

S. No	Media	Solubility (µg/ml)
1	Distilled Water	82.7±.001
2	Phosphate Buffer pH 7.4	166.7±.006
3	Methanol	225.2±.008

Partition coefficient

A major criterion in evaluation of the ability of a drug to penetrate the lipid membrane is its apparent oil/water partition coefficient ($K_{o/w}$). Therefore the lipid solubility of a drug is determined from ($K_{o/w}$).

The partition coefficient of Terbinafine Hydrochloride was determined in n-octanol: phosphate buffer pH 7.4 system. 10 mg of the Terbinafine Hydrochloride was accurately weighed and dissolved in 10 ml of methanol and 10 ml of n-octanol in separating funnel. This mixture was shaken for 10 minutes interval for 1 hour and left it for 24 hours. The two layers were separated out using separating funnel. The aqueous phase was filtered with the help of filter paper and was diluted 100 times with phosphate buffer pH 7.4. The absorbance of aqueous phase was taken at 282 nm using phosphate buffer pH 7.4 as a blank. The partition coefficient value was calculated and compared with literature value and is reported in Table 7.

$P_{o/w} = (C_{oil}/C_{water})$ equilibrium

Table 7 Partition Coefficient of Terbinafine Hydrochloride

S. No	Method	Experimental	Literature value
1	n- octanol partition coefficient	5.12± 0.058	5.2

Drug polymer interaction studies

While formulation topical gels, it is imperative to give consideration to the compatibility of drug and polymer used within the system. It is therefore necessary to confirm that drug is not interacting with polymer under experimental conditions and to calculate shelf life. Desired quantity of drug with specified excipients (polaxomer) were taken in the ratio 1:1 & 1:5 and mixed thoroughly and filled in dried vials. The vials were sealed and kept at 45ºC for two weeks. The vials were examined daily at regular interval for discoloration, clump formulation and liquefaction.

The infrared absorption spectra of physical mixtures of polymer and drug were run for drug excipients compatibility studies between 400 cm^{-1} - 4000 cm^{-1}. The FTIR spectra of, polymers, physical mixtures of drug and polymer are shown in Fig. 7,8.

Table 8 Physical parameters for compatibility studies

Mixtures	Discoloration		Liquefaction		Clump formation	
Ratio polaxomer	1:1	1:5	1:1	1:5	1:1	1:5
	-	-	-	-	-	-

(- no interaction)

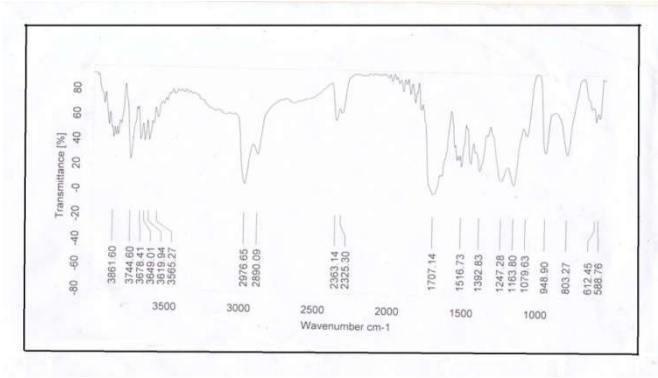

Fig 7 FTIR Spectra of polaxomer

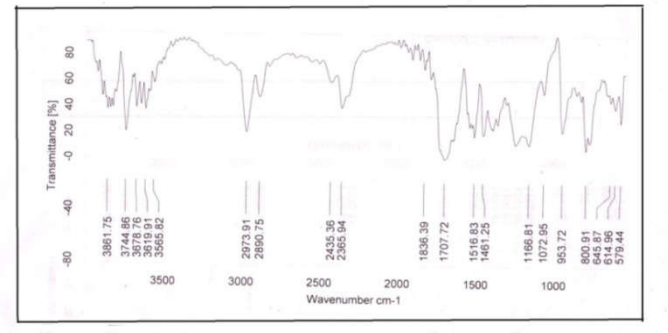

Fig 8 FTIR Spectra of Terbinafine Hydrochloride and polaxomer

METHODOLOGY

Mehod used

Polymerization technique with cold method

Preparation of temperature sensitive Hydrogel

- Distilled water was cooled to 4^0C
- Poloxamer was added slowly with continuous agitation
- The formulation kept in refrigerator at 4^0C until clear solution were obtained
- Terbinafine was dissolved in selected vehicle(methanol) based on the solubility studies
- Dissolved Terbinafine was added to the clear Poloxamer solution
- Missed with glass rod until clear solution were formed

Anti-fungal study

The prepared formula was tested by cup plate method. This method is based on the diffusion of an antibiotic from a cavity through the solidified agar layers of Petri plate. Growth of inoculated micro-organism is inhibited entirely in a circular area "zone" around a cavity containing a solution of the antibiotic for the assay of Terbinafine hydrochloride topical gel, *candida albicans* is used as the test micro-organism and sabouraud[1] agar was used as the medium for growth of test cultures[13,14].

1g of prepared gel (G3) was dissolved in 100 ml of dimethylsulphoxide. By using this stock solution 6, 8, 10 and 12 µg/ml of standard stock solution was prepared. In the same way, standard stock solution of available marketed formulation (Terbif Gel) was prepared[3,15].

The antibiotic assay medium was sterilized by autoclaving and Petri plates were prepared in laminar air flow. Test micro-organism (*candida albicans*) was spread on the surface of Petri plate by spread plate technique. By using flame sterilized cork borer, four cups (or cavities) were bored in each plate at adequate distance from each other. Antifungal solution (0.1 ml to 1ml, depending on the size of cavity) is added in each labelled cavity of plates. All the plates were transferred in refrigerator for proper diffusion of antifungals at 4°C for 1 to 2 h. All the plates were incubated at 32°C-35°C for 24 to 48 h.[4] The zone of inhibition around the cavity was observed. The diameter was measured.

Table 9 Inhibition Zone of the Prepared Topical Gel formulation and Marketed Gel Formulation (Terbif Gel)

Formulation	Antifungal	Microorganism	Concentration	Zone of inhibition (cm)
F3 (Petri plate A)	Terbinafine	*Candida albicans*	C1	2.0±0.032
	Terbinafine	*Candida albicans*	C2	2.4±0.049
	Terbinafine	*Candida albicans*	C3	2.5±0.017
	Terbinafine	*Candida albicans*	C4	2.9±0.041
Marketed Gel (Terbif Gel) (Petri plate B)	Terbinafine	*Candida albicans*	C1	1.4±0.059
	Terbinafine	*Candida albicans*	C2	1.7±0.067
	Terbinafine	*Candida albicans*	C3	2.0±0.084
	Terbinafine	*Candida albicans*	C4	2.4±0.034

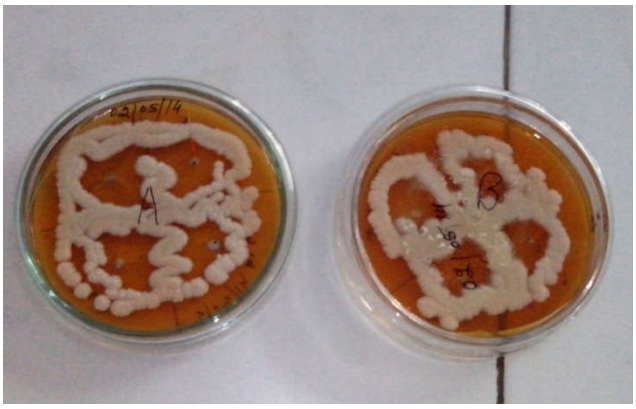

Fig. 9 Inhibition zone of G3 and Marketed Gel (Terbif Gel)

Stability studies

Protocol for Stability Studies of Hydrogels Formulations[1,15]

The product was properly filled in collapsible tubes and was stored according to storage condition.

Table 10 Storage Conditions and Period for Stability Studies

Formulation Code	Storage Condition/Period	
G3	Refrigerated, 2 months	Real Time, 2 months

Testing plan

Topical gel formulation G3 was filled in the collapsible tubes and was stored at the 40^0C/75%RH storage condition.

Table 11 Storage Conditions and Sampling Intervals for Stability Studies

Storage Conditions	Sampling Intervals
Real Time Storage (25^0C/75%RH)	7,14,21,28,35,42,49 and 60 days.
Accelerated (40^0C/75%RH)	7,14,21,28,35,42,49 and 60 days.

Testing and test criteria

The samples were stored and tested in accordance with storage condition and valid test method. The samples were taken out of storage on planned testing date. The parameters to be tested were follows:

- Physical Test-appearance (P.A)
- Drug content (D.C)

Table 12 Stability Profile of Formulation G3 at 25^0C/75% and 40^0C/75%RH Temperature

Time in days	Real Time Storage (25^0C/75%RH)		Accelerated (40^0C/75%RH)	
	D.C	P.A.	D.C	P.A.
0	9.893	+	9.990	+
7	9.87	+	9.944	+
14	9.82	+	9.928	+
21	9.72	+	9.917	+
28	9.63	+	9.872	+
35	9.68	+	9.859	+
42	9.64	+	9.834	+
49	9.60	+	9.814	+
60	9.54	+	9.805	+

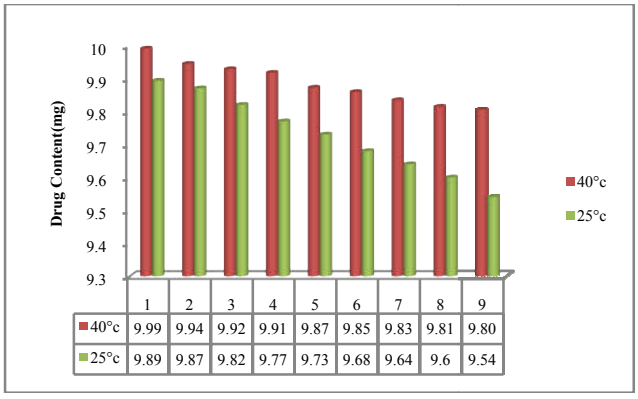

Fig 10 Stability Profile of Formulation G3

Shelf life Determination

The data obtained was fitted into first order equations to determine the kinetics of degradation. Accelerated stability data were plotted according to Arrhenius equation to determines the shelf life at 25ºC.

K =Ae –Ea/RT

$t_{90\%}$ = 0.105/K

Where, K is specific reaction constant,
A is Arrhenius factor,
T is Absolute temperature
R is Gas constant,
Ea is Energy of activation

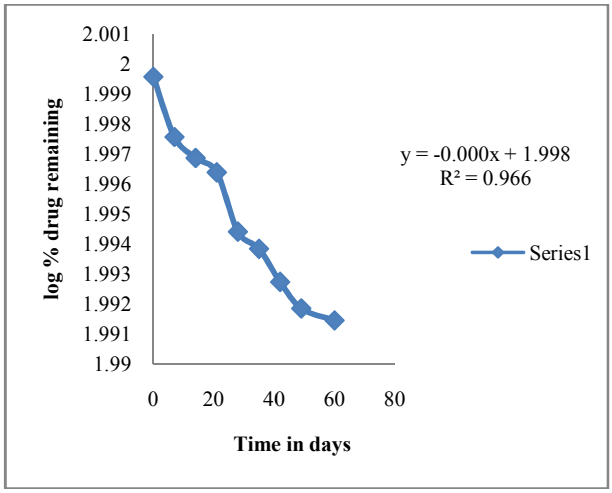

Fig 11 Plot for Drug Degradation at Accelerated condition (40ºC/75 % RH)

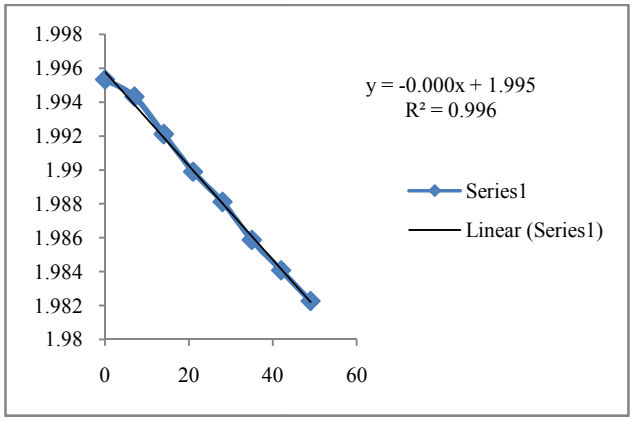

Fig 12 Plot for Drug Degradation at Accelerated condition (25ºC/75 % RH)

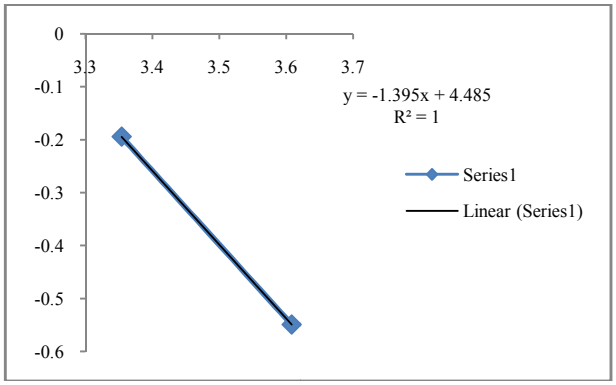

Fig 13 Arrhenius Plot for G3 formulation at 25^0C and 40°C.

RESULTS AND DISCUSSION

Solubility analysis

Terbinafine is poorly water soluble drug about 5.5mg/ml.[1] Since, the aim of our study was to develop hydrogels; the solvent selection was mainly based on drug solubility and also its miscibility with water without precipitating the drug. Based on these characteristics the solubility of terbinafine in different co-solvents was performed and reported using calibration

curve (figure 14) in table 13. The terbinafine exhibited greatest solubility in Transcutol®-P (TCL), propylene glycol (PG) and in polyethyleneglycol400 (PEG 400).So these co-solvents were therefore chosen to be used in the hydroge formulation. Moreover, they are well accepted excipients in pharmaceutical formulations listed under GRAS.

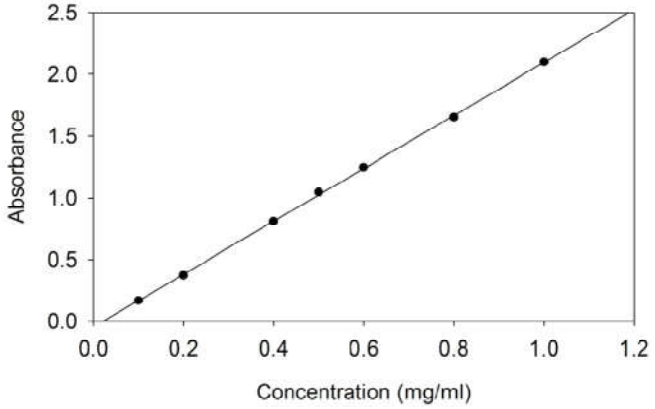

Figure 14 Calibration curve of terbinafine in methanol using UV/VIS spectrophotometer (n=3) r2=0.99

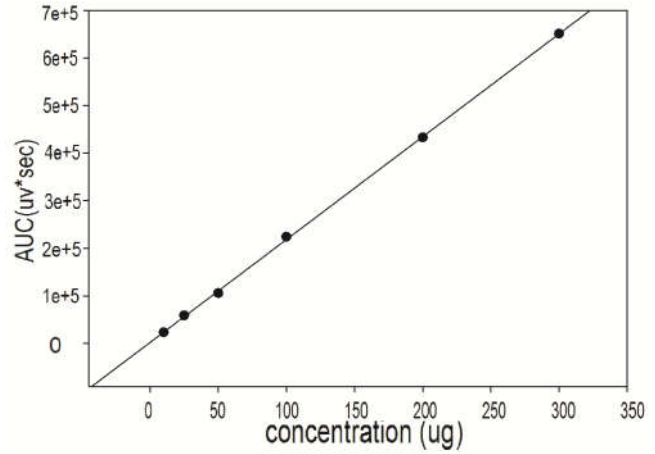

Figure 15 Calibration curve of terbinafine in methanol r2=0.999 using HPLC

Table 13 Solubility of terbinafine in different co-solvents (n=3)

Vehicle	Solubility using UV/VIS (mg/ml)±S.D	Solubility using HPLC (mg/ml)±S.D
Water	6.6±0.3	8.8±0.2
Lauryl glycol	9.8±0.5	10.8±0.1
Ethyl alcohol	73.5±0.7	69.5±0.8
Methanol	92.5±2.2	90±1.7
PEG 400	121.4±1.5	120.4±1.2
Propylene glycol	163.5±1.9	160.3±1.8

Preparation of temperature sensitive hydrogels

In all preliminary studies, method A was used to measure the sol-gel transition temperature. Based on the solubility of terbinafine, three different vehicles (PEG 400, TCL and PG) were selected as co-solvents. To find the formulation showing optimal solgel transition temperature different concentrations of poloxamer and co-solvents were investigated. In all the formulations drug concentration of 1% terbinafine was fixed. First to determine the optimal concentration of poloxamer, different concentrations of poloxamer10%, 15%, 20%, 25%, 30% (w/w) in water were investigated with no cosolvents and terbinafine (table 14).

Table 14 Sol-geltransition temperatures of the aqueous hydrogels with poloxamer alone

Poloxamer (%w/w)	Sol-gel transition temperature (°C) (Method-A)
10%	Gel not formed
15%	39±1.3
20%	39±1.3
25%	14±0.5
30%	9±0.5

The aqueous solutions with poloxamer concentration ranging between 15% to 20% (w/w) poloxamer showed Newtonian or liquid-like behavior at room temperature (25°C) and Non-Newtonian or gel-like behavior at physiological temperature (37°C). In contrast, the formulations with 25% and 30% (w/w) poloxamer concentration showed non-Newtonian or gel-like behavior at both the temperatures. On further investigation the concentration 16% and 17% of poloxamer was fixed which showed optical sol-gel transition temperature (~35°C). The co-solvents along with 1% terbinafine of different concentrations 10%, 20% and 30% were added to the poloxamer solutions to see the effect of co-solvents on the sol-gel transition temperature (Table 15).

Table 15 Effect of co-solvent on sol-gel transition temperature

Poloxamer407NF (%w/w)	Co-solvent	Concentration (%w/w)	Sol-gel transition temperature (°C)±S.D (Method-A)
16	Transcutol	10%	32 ± 0.2
16	Transcutol	20%	43 ±1.5
16	Transcutol	30%	No gelation
17	Transcutol	10%	30 ± 0.5
17	Transcutol	20%	34 ± 0.6
17	Transcutol	30%	No gelation
16	Propylene Glycol	10%	31 ± 0.5
16	Propylene Glycol	20%	30 ±0.5
16	Propylene Glycol	30%	25 ±1.2
17	Propylene Glycol	10%	28 ±0.1
17	Propylene Glycol	20%	25 ±0.3
17	Propylene Glycol	30%	22 ± 0.1
16	PEG400	10%	No gelation
16	PEG400	20%	No gelation
16	PEG400	30%	No gelation
17	PEG400	10%	34 ±0.5
17	PEG400	20%	No gelation
17	PEG400	30%	No gelation

The formulation with vehicle PEG400 showed negative effect by not forming gels, so the PEG 400 was discarded for further investigation. The formulations with vehicles TCL and PG of concentration 30% did not produce gels or produced weak gels with low viscosity. Finally, the formulations with 16% and 17% (w/w) poloxamer along with either co-solvents TCL or PG of concentrations 10% or 20% showed optimal sol-gel transition temperature. The final formulations were free flowing liquids at 20°C storage temperature and formed gels or semi-solids at a temperature of 37°C reported.

Measurement of sol-gel transition temperature

Sol-gel transition temperature I s defined as the temperature at which the liquid or sol phase makes transition to the gel phase. Generally, sol-gel transition for formulations should be in the range of 25-35°C for effective drug delivery.[4,15] Transition temperatures above 37°C result in a liquid formulation at physiological temperature, which in turn can results in rapid clearance of drug. On the other hand, transition temperatures below 25°C, a gel may be formed at room temperature creating application difficulties. Aqueous solutions of poloxamer

exhibit thermoreversible properties. At the sol-gel transition temperature the rheological properties of the system change from Newtonian to Non-Newtonian behavior (Figure 16).In the present study, the transition temperatures of eight formulations were evaluated using rheometer and the results given in table 16.

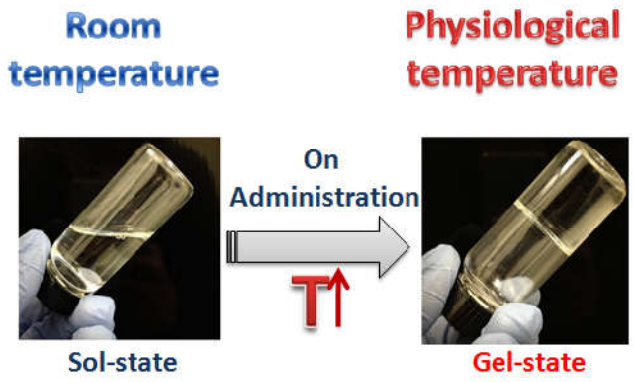

Figure 16 Terbinafine hydrogel behavior at room temperature (~25°C) and physiological temperature (~37°C)

Table 16 The composition and transition temperatures of terbinafine hydrogel formulations (n=3)

Formulation code	Poloxamer407 NF (%w/w)	Transcutol(%w/w)	Propylene Glycol (%w/w)	Sol-gel Transition Temperature (°C)±S.D (Method-B)
F1	16	10	-	31.5±0.3
F2	16	20	-	40.3±1.3
F3	17	10	-	28.1±0.8
F4	17	20	-	31.2±0.3
F5	16	-	10	29.1±0.3
F6	16	-	20	28.2±0.2
F7	17	-	10	26.0±0.5
F8	17	-	20	24.2±0.3

The formulations containing high poloxamer concentration (17%) exhibited lower transition temperatures than formulations with lower poloxamer concentration (16%). This result is in agreement with current literature that describes a decrease in the transition temperature with the increase of poloxamer concentration.[5,16] The type of cosolvent in the formulation also influenced the transition temperature. Formulations containing the co-solvent TCL (F1 to F4) exhibited significantly higher transition temperatures than formulations containing PG (F5 to F8) (Table 16). The addition of cosolvents in the formulation can interfere with the poloxamermicellization and alter the dehydration of hydrophobic PPO blocks. The presence of different co-solvents can therefore modify the transition temperature either by disturbing the micellar packing (increasing the transition temperature as observed with TCL) or by favoring micelle formation (decreasing the transition temperature as observed with PG)[16,17]. This may explain the difference in the transition temperatures observed between the TCL- and PGcontaining hydrogels. Based on the transition temperatures, formulations F1 and F3 to F7 exhibited transition temperatures within the range appropriate for drug delivery.

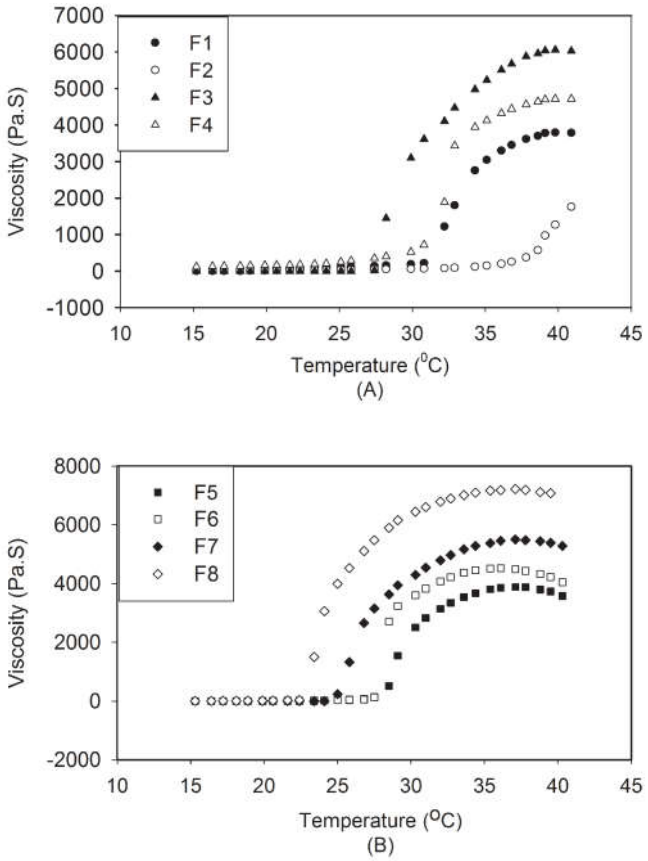

Figure 17 Change in viscosity with respect to temperature (°C) of hydrogels formulations containing co-solvent (A) TCl and (B) PG

Rheological and viscoelastic studies

Rheological studies can be used to predict the *in situ* behavior of semi-solid dosage forms in the body. The flow characteristics of the formulations affect the spreadability and the residence time of the formulation at the application site. The measure of changes in shear stress with shear rates has been used to determine whether the rheological behavior of the samples is Newtonian or Non-Newtonian.[18,19]Poloxamers exhibit pseudoplastic flow when in gel form and upon increasing the shear rate the gel viscosity decreases. As shown in figure 18, the formulations at 370C exhibited a decrease in viscosity with an increase in shear (10 s-1 - 1000s-1), whereas at 20°C no significant change in viscosity was observed (except from formulation F8). This confirms the formulations change in behavior from liquid-like (Newtonian) to gel-like (non-Newtonian) when the temperature increases. Formulation F8 showed shear thinning characteristics at both high (37°C) and low (20°C) temperatures. This may be due to the high concentration of PG and poloxamer in formulation F8which decreased the sol-gel transition temperature (table 16)

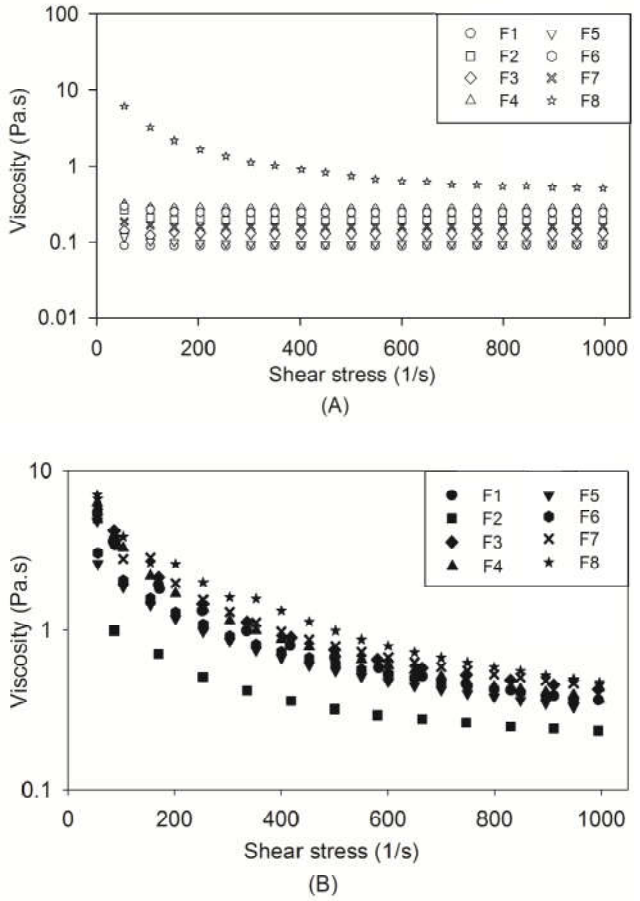

Figure 18 Flow curves of terbinafine formulations at (A) 20°C and (B) 37°C

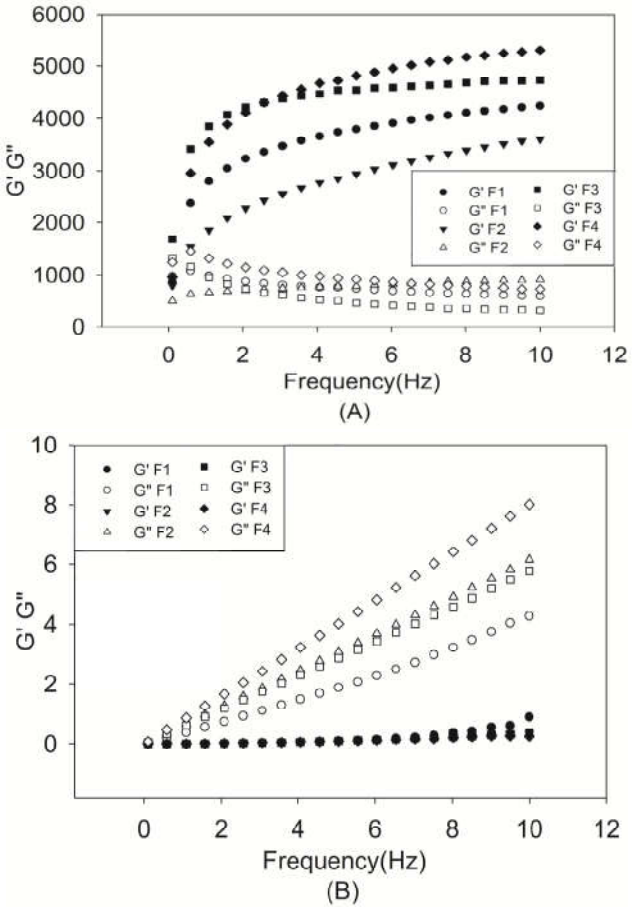

Figure 19 Frequency dependent changes of viscoelastic properties of the terbinafine formulations containing co-solvent TCL; (A) at 37°C and (B) at 20°C

In oscillatory rheometry the effect of sinusoidal shear stress on the viscoelastic properties are measured and two dynamic moduli, i.e. the elastic or storage modulus (G') and the viscosity or loss modulus (G"), are obtained as a function of oscillatory frequency. In this study, the gel structure was examined over a frequency range of 0.1-10Hz, which is a common tested region in rheological studies.[6,20] The elastic modulus G' is a measure of the energy stored and recovered per cycle of deformation, and reflects the solid-like component of elastic behavior.[7]A higher G' than G" value means that under a shearing force, the material is able to store energy and not deform or flow. In contrast, a higher G" value than G' means that under a shearing force, the material is not able to store energy and deforms to flow as liquid(16). It is preferred that the formulation is free flowing at room temperature (25°C) allowing for an easy application. Thus, at room temperature (25°C) the viscosity modulus G" should be higher than the storage modulus G' (G">G'). After administration, the formulation is expected to form gel due to rise in temperature, where G' should be higher than the G" (G'>G"). Figure 19(A) (B) and figure 20(A) (B) shows the frequency dependence at 20°C and 37°C of the elastic modulus (G') and viscosity modulus (G") of all formulations. All the formulations showed gel-like characteristics (G'>G") at 37°C and sol-like character at 20°C (G">G').

However, the formulations with co-solvent PG (F5-F8) showed higher G' values than the corresponding formulations with TCL (F1-F4).This indicates that the formulations with PG are able to form gels with strong cross-linkings and with greater elasticity than formulations with TCL. The greater elasticity of these formulations can be expected to enhance their retention at the application site.[8,18]

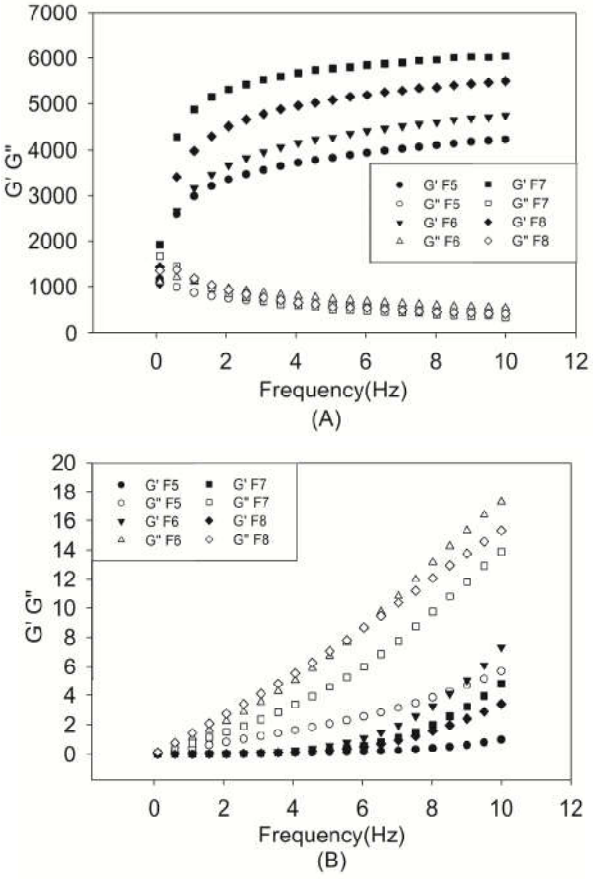

Figure 20 Frequency dependent changes of viscoelastic properties of the terbinafine formulations containing co-solvent PG; (A) at 37°C and (B) at 20°C

The value of loss tangent (tanδ=G''/G'), used as a measure of the relative contribution of viscoelastic properties of the materials. The phase angle (δ) was found higher at 20°C than at 37°C for all formulations (Fig. 21). This lead to the values of loss tangent greater than 1 for all formulations at 20°C indicating the dominance of the viscous component (G''), and was less than 1 at 37°C indicating the prevalence of the elastic component (G').

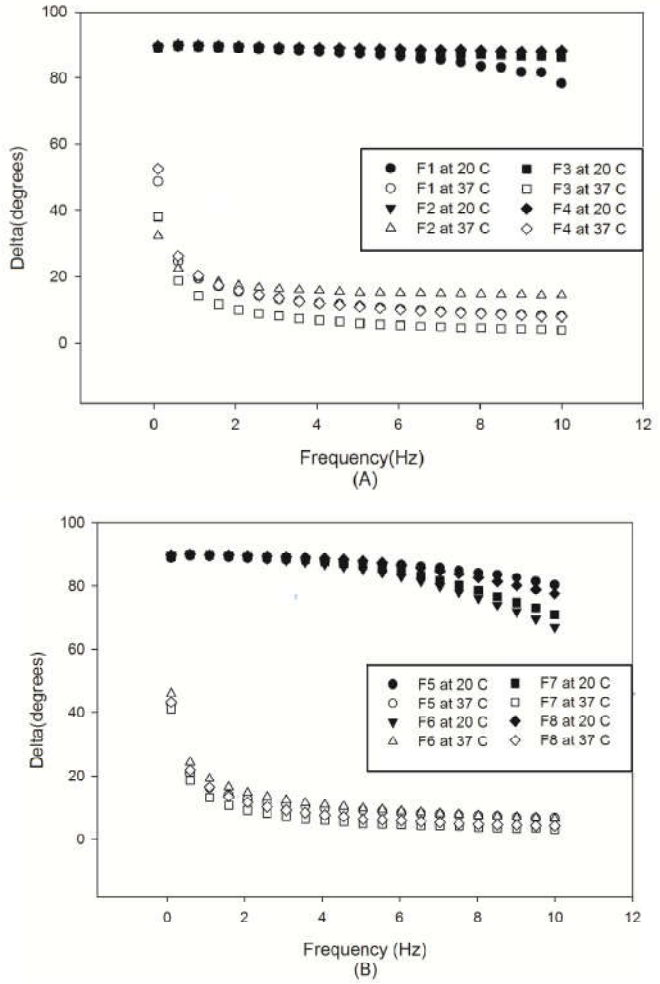

Figure 21 The frequency dependent changes of delta values of terbinafine formulations containing co-solvents (A) TCL (B) PG

Texture profile analysis

A dosage form for topical delivery should possess properties such as good skin spreadability, easy removal of product or dosage form from the package, acceptable viscosity, good bioadhesion and predictable release of active ingredient.[9,22] TPA is used to gather information about the physical gel structure of semi-solid dosage forms, which is useful in predicting the product behavior in different physiological and environmental conditions. In this study, information about the mechanical properties of the gels such as compressibility, hardness and adhesiveness were obtained by the forcetime plots.

The applicability of the gel at the desired site is expressed by hardness, whereas compressibility gives information about the removal of product from the package and its spreadability at the targeted site. The values of hardness and compressibility should be low to easily remove the product from the package and administer with good spreadability. These attributes may contribute to increased patient compliance. All the formulations exhibited low hardness and compressibility at room temperature at which administration is performed. The formulations were tested at physiological temperature (37°C) and results shown in table 17.At 37°C, both the hardness and compressibility values increased as a function of increased poloxamer concentration in the formulation (F3, F4, F7 and F8; Table 17). The difference between formulations containing high and low poloxamer concentrations in hardness and compressibility was found significant *(p>0.05)*. This relationship has also been reported for other types of polymers and is also consistent with the present viscoelastic measurements of the formulations. Above the transition temperature, increased poloxamer concentration in the formulation increases micelle packing and entanglements leading to stronger polymer network.[22]

Table 17 Mechanical properties of terbinafine hydrogel formulations at 37°C (n=3)

Formulation	Hardness (N) ±S.D	compressibility(N. mm)±S.D	Adhesiveness(N.mm) ±S.D
F1	1.42 ± 0.24	9.7 ± 1.3	10.1 ± 0.9
F2	1.45 ± 0.14	9.4 ± 0.4	11.1 ± 0.9
F3	1.66 ± 0.14	11.1 ± 0.6	11.3 ± 0.7
F4	1.83 ± 0.12	12.1 ± 0.3	14.3 ± 0.2
F5	1.52 ± 0.01	10.5 ± 0.5	10.1 ± 0.5
F6	1.46 ± 0.19	9.9 ± 0.7	10.7 ± 0.7
F7	1.69 ± 0.16	12.2 ± 0.5	10.7 ± 0.7
F8	1.71 ± 0.16	11.8 ± 0.7	11.6 ± 0.5

Adhesiveness has been defined as the work required to overcome the attractive forces between the surface of the sample and the surface of the probe in which its cohesive bonds are broken.[10]High adhesiveness values therefore indicate greater adhesion at the tissue surface with subsequent increase in retention time.[10] At 37°C, formulations F4 and F7 with high content of poloxamer demonstrated the significant higher adhesiveness values among the formulations tested with enhanced adhesion to a tissue surface.

Mucoadhesive studies

Mucoadhesive force is defined as the force with which the formulation binds to mucosa or mucous membranes,[23] the higher the value the stronger the formulation binds to the mucosal surface. Mucoadhesive properties are important when prolonged residence time is desirable at mucosal sites to improve absorption and/or reduce rapid drainage from the application site. In this study compressed mucin disks in combination with tensile analysis were used to investigate the *in vitro* mucoadhesive properties of the hydrogels at 37°C. Maximum detachment force and work of adhesion are common indicators to assess the mucoadhesive strength of semi-solid dosage forms.

The results are reported in Table 18. No significant difference *(p<0.05)* in the maximum detachment force was observed between the tested formulations. However, the difference in the work of adhesion values of formulations F2, F4, and F7 were statistically significantly *(p>0.05)*. F2 expressed the lowest work of adhesion, whereas F4 and F7 expressed the highest values. In surface science, work of adhesion is described as the work required to separate the liquid from the solid. It has been proposed that work ofadhesion provides a more reliable and reproducible indicator of the mucoadhesion phenomenon because it represents the total sum of established adhesive joints.[12,24] Mucoadhesive character generally increases with gel strength.[25,26] Due to high polymer concentrations, the swelling and formation of strong and

viscous gels of F4 and F7 at 37°C strengthened the interactions between the formulations and the mucin disks. This observation was consistent with the adhesiveness results. Having transition temperature ~40°C, the low mucoadhesion values of F2 may be due to its proximity to liquid nature *in vitro* at body temperature.

Table 18 Mucoadhesive properties of terbinafine hydrogel formulations at 37°C (n=3)

Formulation	Maximum detachment force(N) ±S.D	Mucoadhesion (mJ) ±S.D
F1	0.22 ± 0.030	0.56 ± 0.03
F2	0.20 ± 0.005	0.28 ± 0.01
F3	0.22 ± 0.001	0.54 ± 0.02
F4	0.23 ± 0.012	0.73 ± 0.05
F5	0.21 ± 0.017	0.48 ± 0.04
F6	0.19 ± 0.004	0.53 ± 0.03
F7	0.22 ± 0.004	0.65 ± 0.06
F8	0.20 ± 0.006	0.50 ± 0.03

In vitro permeation studies

The absorption process of a drug is affected by the nature of the vehicle by influencing the partitioning behavior of the drug between the vehicle and the tissue surface.[27] The *in vitro* permeation studies were performed on cellulose acetate membranes using Franz diffusion apparatus, to investigate how the different gel formulations influence the permeation of the drug from the vehicle. The amount of drug permeated through the membrane was calculated using the calibration curve plotted using HPLC figure 15.

Viscosity is inversely related to the release of active substances from formulation and its penetration through diffusion barriers.[6] Hence, drugs permeate more slowly through viscous vehicles, allowing for prolonged drug release. It was thus expected that formulations containing high poloxamer concentrations (F3, F4, F7 and F8) express low permeability coefficients (K). However, no significant difference in the permeability values was found among the formulations. The flux (J) and permeation (K) of all the formulations were calculated from graphsreported in table 19

Table 19 Results from the *in vitro* permeation studies of the terbinafine hydrogel formulations.

Formulation	Flux J (µg/cm2/h)
F1	18667±1609
F2	20517±1565
F3	16544±1107
F4	16512±2253
F5	18116±1231
F6	17770±1137
F7	15900±1753
F8	14637±1553

In vitro antifungal activity

The antifungal activity of the hydrogel formulations was assessed using the disk diffusion method. The results are presented in table 20. The test for zone of inhibition measures the ability of an antimicrobial product to prevent growth of microorganisms in a rapid manner. The zone of inhibition is defined as the clear area around the paper disk containing an antimicrobial agent on an agar surface.[28]The clear region is an indication of the absence, or the effective inhibition, of microbial growth by the antimicrobial product. The size of the region is related to the intensity of antimicrobial activity. The zone of inhibition measured for the standard disks showed that the selected *Candida* species ATCC 90028 is susceptible to the dose of terbinafine administered (Table 20).No significance difference*($p<0.05$)* in the zone of inhibition was observed betweenthe sample disks and the standard disks (Figure 9). This indicates that all terbinafine containing hydrogel formulations expressed antifungal effect comparable to terbinafine standard and that the gel vehicle did not influence the antifungal activity of terbinafine.

Table 20 Antifungal activity of 25µg terbinafine standard and different terbinafine hydrogel formulations containing 25µg terbinafine/20µl formulation (n=3).

Formulation	Zone of Inhibition (mm) ± S.D
F1	33.0±0.4
F2	33.4±0.5
F3	33.3±1.3
F4	33.9±0.8
F5	32.7±0.5
F6	32.4±0.6
F7	33.6±0.1
F8	33.6±0.4
Standard Terbinafine 25µg	33.6±0.3

CONCLUSION

In this study, different poloxamer concentrations 10 to 30 % (w/w) were evaluated to produce a hydrogel. The concentrations between 15 to 20% (w/w) poloxamer showed Newtonian or liquid-like behavior room temperature (25°C) and non-Newtonian or gellike behavior at physiological temperature (37°C). 1% terbinafine the active ingredient of the formulation is dissolved in co-solvents Transcutol-P, propylene glycol and PEG 400 and poloxamer as polymer. PEG 400 did not show desired effect so discarded from study. Eight different formulations (F1-F8) were selected for further investigation.

All formulations except F8exhibited Newtonian (liquid-like) behavior at 20°C and non- Newtonian (gel-like) behavior at 37°C. F8, containing high poloxamer and PG concentrations, exhibited shear thinning behavior at both 20°C and 37°C. Formulations containing high poloxamer concentrations (F3, F4, F7 and F8) formed stronger and more viscous gels with subsequent higher values in hardness and compressibility at physiological temperature. Moreover, formulations F4 and F7 rendered gels with highest adhesiveness and the most mucoadhesive character. These properties are advantageous to prolong residence time and reduce leakage of the dosage form, when applied on mucosal layers.

In-vitro performance of the hydrogels, No significant differences in the permeability and antifungal activity of terbinafine were observed between the formulations. The type and amount of co-solvent included in the formulation influenced the transition point of the thermo sensitive systems; TCL increased the transition temperature, while the opposite is true for PG. Based on the transition temperature, and the rheological, mechanical and mucoadhesive properties, formulations F4 and F7, containing 17% poloxamer and 20% TCL and 10% PG, respectively, seemed to be the most promising thermosensitive systems for efficient delivery of terbinafine.

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