IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
September 2017 Vol.:10, Issue:2

Formulation and Evaluation of Terbinafine Hydrochloride Loaded Microsponge Based Gel for Topical Sustained Delivery

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Anju V.S*, Dr. M. A. Kuriachan

Department of Pharmaceutics, Mar Dioscorus College of Pharmacy, Thiruvananthapuram, Kerala.

Submission: 23 August 2017
Accepted: 29 August 2017
Published: 30 September 2017

Keywords: Terbinafine hydrochloride, Microsponge, Carbopol, Quasi-emulsion solvent diffusion

ABSTRACT

The objective of present work was formulation and evaluation of Terbinafine hydrochloride loaded microsponge based gel for topical sustained delivery. Terbinafine hydrochloride is an orally and topically active drug belonging to allylamine class of antifungal effective against dermatophytes and Candida group of fungi. The proposed work involves the formulation of Terbinafine hydrochloride microsponges by quasi-emulsion solvent diffusion method using different concentrations of ethyl cellulose as polymer and the drug loaded microsponges equivalent to 1%w/w was incorporated into carbopolgel. The compatibility of drug with excipients was studied by FTIR spectroscopy. Physical examination, particle size, surface morphology and drug entrapment efficiency of prepared microsponges were evaluated. Physical appearance, pH, drug content, spreadability, skin irritancy, in-vitro drug release, rheological studies, antimicrobial study and stability studies of drug loaded microsponge gel formulations were performed. It was found that the concentration of polymer and the emulsifying agent influenced the particle size, production yield, and drug release profile of the microsponge formulations. The optimum sustained release of drug around a period of 10 h was shown by formulation F2. The ‘n’ value of optimized formulation and drug loaded plain gel indicated that the drug release follows anomalous non-Fickian release. From the rheological studies, it was observed that the optimized formulation follows non-Newtonian flow and shear thinning or pseudoplastic behaviour. It was confirmed from the stability studies that the optimized formulation remained stable at 40oC and 75% relative humidity.

INTRODUCTION

[1]The major challenge to the pharmaceutical industry is to control the rate of delivery of active pharmaceutical ingredient to a predetermined site in the human body. So researchers focused on designing different controlled release drug delivery systems to improve efficacy and patient compliance. Controlled release of drugs onto the epidermis with assurance that the drug remains primarily localized and does not enter the systemic circulation in significant amounts. It is an area of research that has only recently been addressed with success.[2] Conventional formulations such as creams, ointments, gels, lotions etc for topical treatment are proposed to act on the outer layer of skin. On topical application, they release the active ingredient and get rapidly absorbed.

[3]An extended release dosage form is a formulation in which the drug-release characteristics of time course and/or location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage forms such as solutions, ointments, or promptly dissolving dosage forms. Examples of extended-release dosage forms include controlled-release, sustained-release, and long-acting drug products. Controlled release systems offer prolonged release at a specific predetermined controlled rate, i.e. drug release is definite per unit time. Whereas, sustained release systems refer to systems offering prolonged drug release, not necessarily with specific rate, i.e. drug release is not definite per unit time. The objective of both the controlled release and sustained release drug delivery systems is to control the drug delivery to ensure safety and enhance the efficacy of drug with improved patient compliance.

Microsponges are polymeric delivery systems composed of porous microspheres of an inert polymer that can entrap active ingredients and control their delivery rate. [4]They are tiny true sponge like spherical particles that consist of myriads of interconnecting voids within a non-collapsible structure with large porous surface. The size of these microsponges can be varied, usually from 5 to 300µm indiameter.[5]Microsponges are designed to deliver a pharmaceutically active ingredient efficiently at minimum dose and also to enhance stability, reduce side effects, and to modify drug release profile. The microsponge technology can be utilized in avariety of formulations like creams, ointments, lotions etc, but is more frequently manufactured as gels. Once applied on the skin, microsponges slowly release the active agent through the porous surface in a controlled manner.

Terbinafine hydrochloride is an orally and topically active drug belonging to allylamine class of antifungals effective against dermatophytes and *Candida* group of fungi. It is topically used for superficial skin infections such as jock itch (tineacruris), athlete's foot

(tineapedis) and other types of ringworm (tineacorporis) infections.

The aim of proposed work was to formulate Terbinafine hydrochloride loaded microsponge based gel to sustain its topical action and to reduce its side effects, hence to increase the patient compliance.

MATERIALS AND METHODS

MATERIALS

Terbinafine hydrochloride was supplied from Yarrow Chem Products, Mumbai. All other excipients and solvents used were of analytical or pharmaceutical grade.

METHODS

- **Compatibility studies using FT-IR Spectroscopy**

The pure drug, drug and polymer were prepared and scanned from 4000-400cm^{-1} in FTIR spectrophotometer. The FT-IR spectrum of the obtained sample of drug and polymer were compared with the standard functional group frequencies of Terbinafine hydrochloride, ethyl cellulose, carbopol 934 respectively. The compatibility between the drug, polymer were evaluated using FTIR peak matching method.

- **Preparation of Standard Calibration Curve of Terbinafine hydrochloride**[7]

Accurately weighed 10mg of Terbinafine hydrochloride was taken in a 100ml standard flask. Added few ml of ethanol to dissolve the drug and made up to the volume with acetate buffer pH 5.5 to get a stock solution of concentration 100µg/ml. From this stock solution, aliquots of 0.5, 1, 1.5, 2, 2.5ml of solutions were transferred into separate 10ml standard flasks and made up to the volume with acetate buffer pH 5.5 to get concentrations of 5, 10, 15, 20, 25 µg/ml respectively.[7] The absorbance of resultant solutions was measured at 282nm by UV spectrophotometer. A graph of concentration Vs absorbance was plotted.

- **Preparation of Terbinafine hydrochloride microsponges by quasi-emulsion solvent diffusion method**

The internal phase consists of accurately weighed amount of Terbinafine hydrochloride and ethyl cellulose dissolved in dichloromethane.The external phase which consists of polyvinyl alcohol dissolved in warm water was used as emulsifying or stabilizing agent. The internal phase was gradually added into external phase and stirred mechanically at 1000rpm for 2h at room temperature to remove the solvent dichloromethane from the mixture. Microsponges formed were filtered and dried at room temperature and stored in a tightly closed container.

Table 1: Formulation of Terbinafine hydrochloride loaded microsponges

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Terbinafine hydrochloride (mg)	250	250	250	250	250	250	250	250
Ethyl cellulose (g)	1	0.8	0.5	0.3	1	0.8	0.5	0.3
Dichloro methane (ml)	8	8	8	8	8	8	8	8
Poly vinyl alcohol (% w/v)	0.75	0.75	0.75	0.75	0.5	0.5	0.5	0.5

- **Preparation of carbopol gel base**

Accurately weighed amount of carbopol934 was taken and soaked in water for

24h for complete swelling of polymer.

- **Preparation of drug loaded plain carbopol gel**

To the weighed amount of carbopol gel base, 1%w/w of Terbinafine hydrochloride drug was added. PEG 400 and ethanol was added as penetration enhancers. Benzyl alcohol was added as preservative. Triethanolamine was added dropwise with gentle stirring using a homogenizer for adjusting the pH.

- **Preparation of Terbinafine hydrochloride loaded microsponge based gel**

To the weighed amount of carbopol gel base, Terbinafine hydrochloride loaded microsponges equivalent to 1 %w/w were uniformly dispersed. PEG 400 and ethanol was added as

penetration enhancers. Benzyl alcohol was added as preservative. Triethanolamine was added dropwise with gentle stirring using a homogenizer for adjusting the pH.

Table 2: Formulation of Terbinafine hydrochloride loaded microsponge based gel

Ingredients	Quantity
Terbinafine hydrochloride microsponges	1% w/w
Carbopol 934	1 % w/v
Ethanol (ml)	1
PEG 400 (ml)	0.5
Benzyl alcohol (ml)	0.02
Triethanolamine	q.s

- **Characterization and Evaluation of Terbinafine Hydrochloride Loaded Microsponges**

1. Physical properties

The prepared Terbinafine hydrochloride microsponge formulations were inspected visually for their colour and appearance.

2. Particle size analysis[8]

The mean particle size of Terbinafine hydrochloride loaded microsponges was determined using an optical microscope. The microscope was fitted with a stagemicrometer to calibrate the eyepiece micrometer. The particle diameter of around 30 particles was measured in a field.The average particle size was determined using the following formula:

$$D_{mean} = \sum nd / \sum n$$

Where, n = number of microsponges observed and d =mean size range. Each formulation was observed three times and average of three trials was calculated.

3. Scanning electron microscopy

For the evaluation of surface morphology of microsponges, the sample was analyzed in scanning electron microscope after preparing the sample by lightly sprinkling on a double adhesive tape stuck to an aluminium stub. The stubs were then coated with platinum. The

stub containing the coated sample was placed in scanning electron microscope (JEOL JSM 6380LA, Japan). The samples were then randomly scanned and photomicrographs were taken at the acceleration voltage of 20kV. From the resulting image, average particle size was determined.

4. Production yield (%)[9]

All the prepared microsponge formulations were accurately weighed and the weight was recorded. The production yield of the microsponges was then determined using the following equation:

$$\text{Production yield (\%)} = \frac{\text{Practical mass of microsponges}}{\text{Theoretical mass (polymer + drug)}} \times 100$$

5. Drug entrapment efficiency (%)[8]

Accurately weighed quantity of prepared Terbinafine hydrochloride loaded microsponges were taken and crushed in a mortar and pestle. 5ml of ethanol was added and transferred contents to a 100ml standard flask and made up to the volume with acetate buffer pH 5.5. Kept aside for 1h with frequent shaking for extracting the drug from the microsponges. Then it was filtered and the absorbance of filtrate was measured at 282nm after suitable dilutions. The drug content was calculated from the calibration curve and expressed as actual drug content in microsponge. The drug entrapment efficiency (%) of the microsponges was calculated according to following equation:

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Experimental drug loading}}{\text{Theoretical drug loading}} \times 100$$

- **Evaluation of Terbinafine hydrochloride loaded microsponge based gel**

1. Physical Examination

The prepared microsponge based gels were inspected visually for their colour, homogeneity and consistency.

2. Determination of pH[10]

One gram of microsponge gel was dissolved in 100 ml of distilled water and stored for two hours. Then, pH was measured by digital pH meter. The measurement of pH of each formulation was done in triplicate and average values were calculated.

3. Drug content (%)

1 g of prepared terbinafine hydrochloride loaded gel formulation containing drug equivalent to 10mg was extracted with 30ml of ethanol. The volume was made up to 100ml with acetate buffer 5.5. The solution was filtered. The absorbance of the resulting solution was measured at 282nm using UV spectrophotometer after suitable dilutions. The drug content of drug loaded plain gel was also determined in the same manner. The drug content of the formulation was determined using the following equation:

$$\% \text{ Drug content} = \frac{\text{Actual concentration of drug in the formulation}}{\text{Theoretical concentration of drug}} \times 100$$

4. Skin irritancy study[11]

Skin irritation test was performed for the final microsponge gel formulations on human volunteers to find out any irritation problems which could make it unsuitable for topical use. About 1 g of final formulation to be tested was applied to the sensitive part of the skin (like wrist portion of the hand). The site of application was inspected for irritancy, erythema and edema.

5. Spreadability studies[12]

2g of the formulation was placed on a ground glass slide fixed on a wooden block. The gel formulation was sandwiched between this slide and the second slide having same dimensions. Second slide was provided with a hook. Measured quantity of weight (30g) was placed in a pan attached to the pulley with the help of hook. Time (in seconds) required by the top slide to separate from ground slide was noted. Shorter the interval, better the spreading coefficient.

$$\textbf{Spreadability} = M \times L/T$$

Where, M = weight tied to the upper slide (30g)

L =length of glass slide (5cm)

T =time taken in seconds

6. *In- vitro* drug release studies[13]

In- vitro release study of terbinafine hydrochloride loaded microsponge based gel was carried out by using Franz diffusion cell. The formulation was taken in the donor compartment and acetate buffer pH 5.5 was taken in the receptor compartment. The cellophane membrane, previously soaked overnight in the diffusion medium (acetate buffer pH 5.5) was placed between the donor and receptor compartment. 1g of the formulation was spread uniformly on the cellophane membrane, which is in contact with the receptor medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer with continuous stirring and the temperature of the medium was maintained at 37± 0.5^{o}C. At specific intervals, 2ml of sample was withdrawn from the receptor compartment and replaced with an equal volume of acetate buffer pH 5.5. The *in-vitro* drug release of drug loaded microsponge based gel was compared with drug loaded plain gel. Absorbance of the sample was determined after suitable dilutions at 282nm using UV-visible spectrophotometer.

7. Kinetics of *in-vitro* drug release[14]

The results obtained from *in-vitro* release studies were attempted to be fitted into various mathematical models as follows:

1. Cumulative percent drug released Vs. Time (Zero order kinetics)
2. Log cumulative percent drug retained Vs. Time (First order kinetics)
3. Cumulative percent released Vs. Square root of Time (Higuchi model)
4. Log cumulative percent drug released Vs. Log Time (Korsemeyer-Peppas model)

In Peppas model, the value of ‘n’ characterizes the release mechanism of drug as described in

Table 3: Interpretation of diffusional release mechanism

Release exponent (n)	Diffusion release mechanism
0.45	Fickian diffusion
0.45 <n<0.89	Anomalous(Non-Fickian) diffusion
0.89 - 1.0	Case II transport (Zero order release)
>1.0	Super case II transport

8. Rheological studies[15]

Dynamic oscillation frequency sweep test was used to determine the capability of the gel to resist structural changes and the visco-elastic properties of the gel under the increased frequency. The rheological properties of the optimized formulation of Terbinafine hydrochloride loaded microsponge based gel(F2) was determined using Rheometer MCR51 by frequency sweep analysis. About 0.5g of the formulation was applied to the plate and left for equilibrium. Measurements were made at fixed temperature 25^{o}C at angular frequency (shear rate) ranging from 100-0.631 rad/s.

Using the data obtained from rheological studies, following plots were made:

1. Log Angular frequency VsLog Storage modulus
2. Log Angular frequency VsLog Loss modulus
3. Log Angular frequency VsLog Complex viscosity

Storage modulus in visco-elastic materials is the measure of stored energy representing the elastic behaviour. Loss modulus is related to the amount of energy lost due to viscous flow. Complex viscosity, when plotted as a function of angular frequency, can be correlated to shear viscosity as a function of shear rate.

9. Evaluation of antifungal activity by disk diffusion method[16]

The microorganism used in this study was fungus *Candida albicans*. An antimicrobial assay was performed by using the Kirby-Bauer disk diffusion agar plate method. Agar plates were prepared by pouring freshly prepared agar medium to the sterilized petri dishes after

autoclaving. The microbial suspension of *Candida albicans* was applied onto the solidified agar by using sterile cotton swabs and was allowed to dry for 10 minutes. Formulated gel containing drug loaded microsponges impregnated discs were aseptically transferred onto the inoculated agar plates and left to be incubated for 2 days. The clear zones of inhibition around the test sample disc were shown for any indication of antimicrobial activity. All assays were carried out in triplicate.

10. Stability studies[17]

Stability studies were carried out on the optimized formulation according to ICH guidelines. The optimized formulation was packed in a tightly closed containers and was stored in ICH certified stability chamber maintained at 40±2^{o}C and 75 ±5% RH for one month. The formulation was evaluated before and after at periodic intervals for change in appearance, pH, drug content and *in-vitro* drug release.

RESULTS AND DISCUSSION

- **Compatibility studies**

The FT-IR spectrum of Terbinafine hydrochloride is shown in **Figure 1**, which complies with standard functional group frequencies.

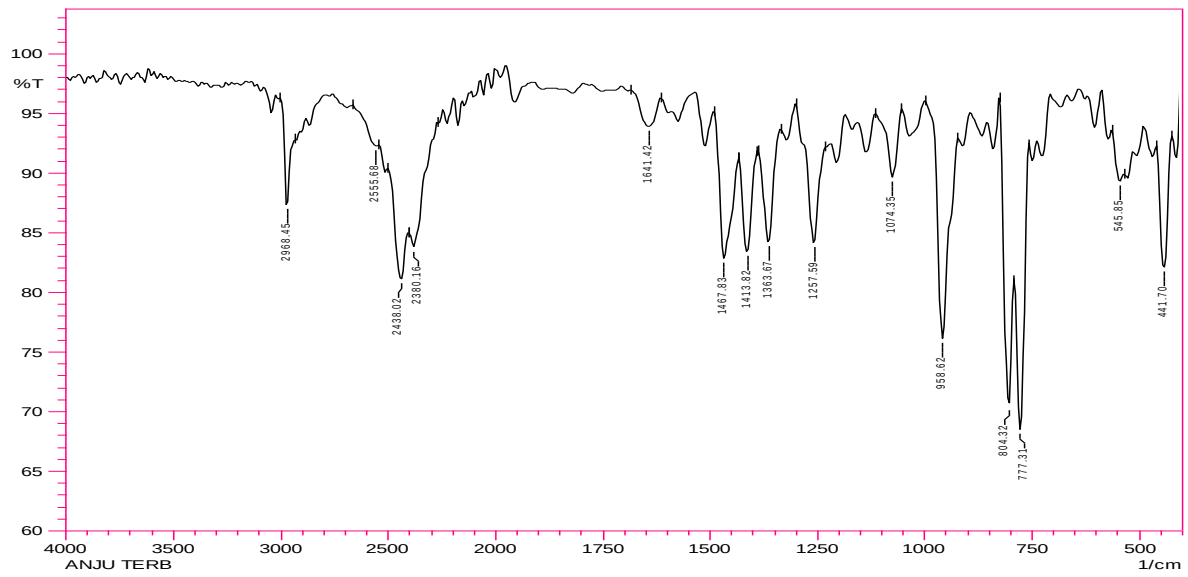

Figure1: FTIR spectrum of Terbinafine hydrochloride

Table 4: IR frequencies of Terbinafine hydrochloride

Functional group	Characteristic wave number	Terbinafine hydrochloride-observed wave number
CN stretching	2500-2400	2438.02
CH bending	1600-1400	1467.83
Enes	950-900	958.62
-C- |	1300-1250	1257.59

- **Compatibility between drug and polymer**

The FTIR spectrum of combination of Terbinafine hydrochloride with excipients is shown in **Figure 2.**

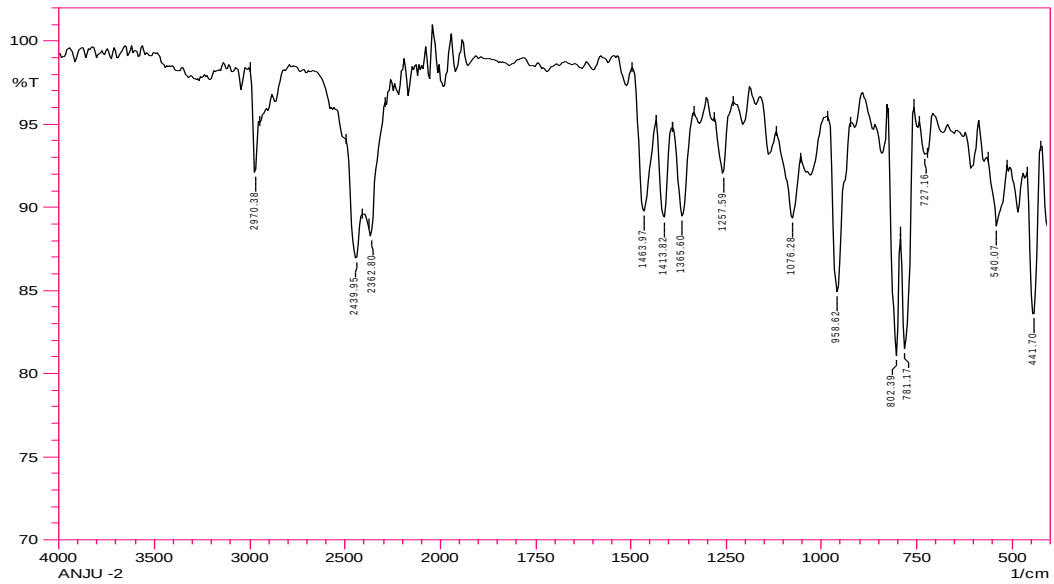

Figure 2: FTIR spectrum of physical mixture Terbinafine hydrochloride + Ethylcellulose + Polyvinyl alcohol + Carbopol934

Table 5: IR frequencies of Terbinafine hydrochloride with other excipients

Functional group	Characteristic wave number	Terbinafine hydrochloride- observed (wave number)	Terbinafine hydrochloride-excipient mixture (wave number)
CN stretching	2500-2400	2438.02	2439.95
CH bending	1600-1400	1467.83	1463.97
Enes	950-900	958.62	958.62
-C-	1300-1250	1257.59	1257.59

The peaks analyzed in **Table 5** indicate that most characteristic frequencies of functional group of Terbinafine hydrochloride which are CN stretching, CH bending, enes, C- etc were found unchanged. This showed that the Terbinafine hydrochloride remained unaffected by the excipients used. So it could be concluded that there was no major interaction between drug and excipients used.

- **Preparation of standard calibration curve of Terbinafine hydrochloride**

The calibration curve was found to be linear in the range of 5-30 µg/ml at λ_{max} 282nm.

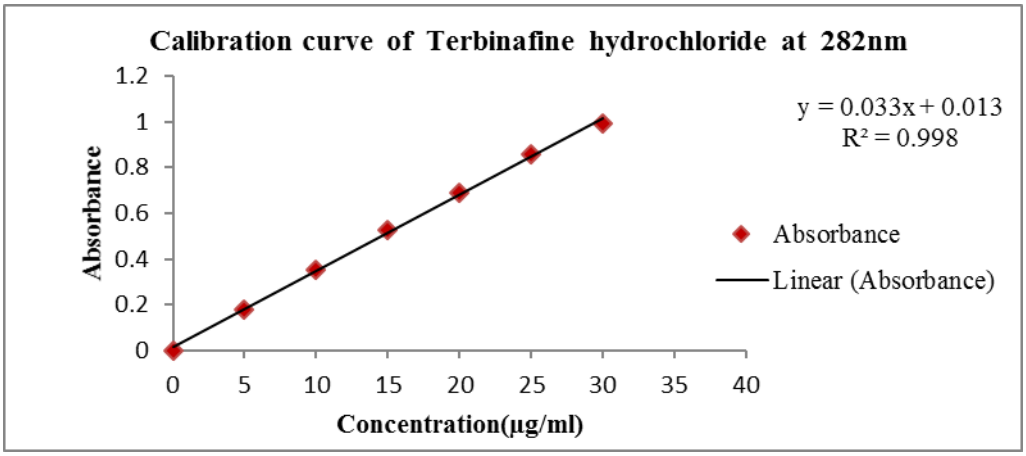

Figure 3: Standard calibration curve of Terbinafine hydrochloride in acetate buffer 5.5

- **Formulation of Terbinafine Hydrochloride loaded microsponge based gel**

Terbinafine hydrochloride loaded microsponges were prepared by quasi-emulsion solvent diffusion method, because of its reliability, simplicity and reproducibility, and reduced solvent toxicity. In this method, the formation of microsponges could be prepared by the rapid diffusion of dichloromethane into the aqueous medium containing polyvinyl alcohol that might reduce the solubility of the polymer in the droplets, since the polymer was insoluble in aqueous media. The instant mixing of the internal-phase and external–phase at the interface of the droplets, induce precipitation of the polymer, thus forming a shell enclosing the inner-phase and the dissolved drug. The finely dispersed droplets of the polymer solution of the drug were solidified in the aqueous phase via diffusion of the solvent.

- **Characterisation and evaluation of terbinafine hydrochloride loaded microsponges**

1. Physical properties

All the prepared Terbinafine hydrochloride loaded microsponge formulations were white in colour, free-flowing in nature and had rigid spherical structure. F1 formulation had a comparatively hard structure because of increased polymer concentration. Minimum concentration of external phase is required to bring about the formation of uniform and stable microsponges. Insufficient concentration of emulsifying agent may produce unstable microsponges. **Figure 4** shows the prepared microsponge F2.

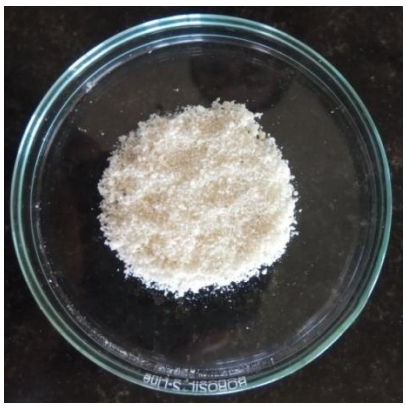

Figure 4: Prepared microsponge F2

2. Particle size analysis

The mean particle size of Terbinafine hydrochloride loaded microsponges ranged from

87-103µmas shown in **Table6**. It was found that, when concentration of polymer increases, the mean particle size of the microsponge also increases. This may be attributed to the higher viscosity of the internal-phase, thus increasing the chances of formation of bigger particles and faster diffusion of the solvent.

3. Scanning electron microscopy

The SEM image is shown in **Figure5**. The SEM images showed that the surface of prepared microsponges was spherical in shape and uniform in size and its surface was porous in nature. The pores were induced by the diffusion of the volatile solvent (dichloromethane) from the surface of the microparticles. Based on SEM studies, the mean particle size of microsponges was found to be 100 µm.

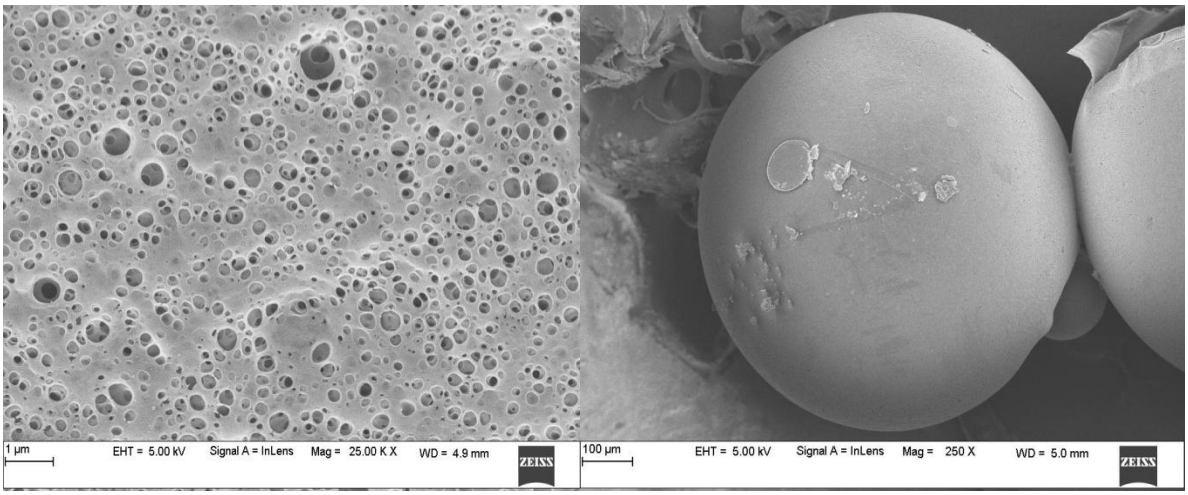

Figure5: SEM images of microsponge F2

4. Production yield (%)

The production yield of Terbinafine hydrochloride loaded microsponges was found to be in the range of 46.66- 77.86 % as reported in **Table 6.** When the concentration of polymer was increased, the production yield of microsponges was also found to be increased. This may be due to higher amount of polymer, thus resulting in an increase in total mass of the microsponges.

5. Drug entrapment efficiency (%)

The drug entrapment efficiency (%) of Terbinafine hydrochloride ranged from 42.31–60.82% as shown in **Table 6**. The results of drug entrapment efficiency (%) showed that, with

increase in polymer concentration, the drug entrapment efficiency (%) was also increased. The increase in drug entrapment efficiency with increase in polymer concentration may be due to the sufficient amount of polymer being available for the drug to be entrapped.

Table 6: Mean particle size, % production yield, % drug entrapment efficiency

Formulation	Mean particle size (µm)	% Production yield	% Entrapment efficiency
F1	103	77.86 ±0.15	60.82 ± 0.44
F2	100	73.65 ± 0.18	59.05 ± 0.48
F3	95	65.77 ± 0.21	51.43 ± 0.54
F4	89	51.51 ± 0.13	45.57 ± 0.55
F5	102	71.73± 0.19	56.60 ± 0.41
F6	97	70.47 ± 0.17	55.00 ± 0.43
F7	92	61.77 ± 0.18	49.57 ± 0.50
F8	87	46.66 ± 0.11	42.31 ± 0.52

- **Evaluation of terbinafine hydrochloride loaded microsponge based gel**

1. Physical examination

All the prepared microsponge based gel formulations were consistent, viscous with a smooth and homogenous appearance. All the formulations appeared like white colored microsponges suspended in transparent gel base.

2. Determination of pH

The pH of all the Terbinafine hydrochloride microsponge based gel formulations was found to be in the range of 5.7-6.2 and pH of drug loaded plain gel was 5.8 as shown in **Table 7**.

3. Drug content (%)

The percentage drug content of drug loaded plain carbopol 934 gels, as well as microsponges enriched gel was found to be in the range of 89.18 - 92.10 % as shown in **Table 7**. From this, it was found that the drug remains in entrapped form in microsponges and uniformly distributed into the gels.

4. Skin irritancy study

All the Terbinafine hydrochloride microsponge based gel formulations were tested for skin irritancy. No formulations showed irritation, edema and erythema when applied on skin.

5. Spreadability studies

Spreadability of the formulations is shown in **Table 7.** All the formulations showed good spreadability. Formulation F2 and drug loaded plain gel showed a better spreadability of 9.37g.cm/sec and 10 g.cm/sec respectively compared to other formulations.

Table 7: pH, % drug content, Spreadability

Formulation	pH	% Drug content	Spreadability (g.cm/sec)
F1	5.7 ± 0.03	91.69 ± 0.15	8.82
F2	5.8 ± 0.02	91.12 ± 0.16	9.37
F3	5.7 ± 0.04	90.02 ± 0.11	8.82
F4	5.9 ± 0.02	89.72 ± 0.14	8.33
F5	5.8 ± 0.05	90.31 ± 0.10	8.82
F6	5.9± 0.04	90.28 ± 0.11	9.37
F7	6.0± 0.12	89.65 ± 0.12	8.33
F8	6.2 ± 0.06	89.18 ± 0.13	8.82
Drug loaded plain gel	5.8 ± 0.07	92.10 ± 0.14	10

6. *In-vitro* drug release study

The *in-vitro* drug release studies were carried out using Franz diffusion cell for 10h.The percentage of cumulative drug released from the formulations F1-F4 were tabulated in**Table8** and F5-F8 were tabulated in

Table 8: Percentage cumulative drug release data for formulations F1-F4

Time (h)	F1 %CDR	F2 %CDR	F3 %CDR	F4 %CDR
0	0	0	0	0
1	9.02 ± 0.32	11.97 ± 0.11	10.12 ± 0.55	10.37 ± 0.41
2	22.93 ± 0.15	24.48 ± 0.12	23.72 ± 0.21	21.34 ± 0.45
3	27.84 ± 0.13	35.42 ± 0.37	29.61 ± 0.17	27.48 ± 0.34
4	36.35 ± 0.24	46.16 ± 0.29	38.55 ± 0.23	38.66 ± 0.21
5	45.06 ± 0.15	58.64 ± 0.61	47.45 ± 0.32	45.74 ± 0.11
6	57.43 ± 0.56	67.38 ± 0.51	56.34 ± 0.41	53.7 ± 0.20
7	66.8 ± 0.22	75.89 ± 0.26	68.21 ± 0.54	64.23 ± 0.58
8	73.33 ± 0.24	80.44 ± 0.34	74.14 ± 0.62	72.2 ± 0.66
9	80.1 ± 0.26	84.38 ± 0.22	80.38 ± 0.53	78.26 ± 0.56
10	86.67 ± 0.31	89.80 ± 0.14	87.26 ± 0.41	89.58± 0.17

CDR: cumulative drug release

Table 9: Percentage cumulative drug release data for formulations F5-F8 and drug loaded plain gel

Time (h)	F5 %CDR	F6 %CDR	F7 %CDR	F8 %CDR	Drug loaded plain gel
0	0	0	0	0	0
1	8.15± 0.63	9.97± 0.11	10.45± 0.34	10.21± 0.22	20.11± 0.55
2	13.42± 0.36	15.45± 0.28	18.57± 0.19	21.38± 0.55	36.45± 0.41
3	18.88± 0.10	21.86 ± 0.10	24.52± 0.16	27.16± 0.67	52.24± 0.36
4	30.68± 0.11	34.91± 0.10	36.14± 0.62	39.45± 0.20	70.89± 0.25
5	43.22 ± 0.55	46.65± 0.21	48.22± 0.36	45.07± 0.81	83.09± 0.12
6	52.49± 0.24	55.71± 0.15	57.66± 0.15	58.32± 0.73	96.65± 0.65
7	63.75± 0.16	65.78 ± 0.48	68.36± 0.23	69.81± 0.13	-
8	64.70 ± 0.31	66.83± 0.11	69.34± 0.37	70.62± 0.10	-
9	70.64 ± 0.18	73.13± 0.61	72.89± 0.11	75.82± 0.15	-
10	73.56 ± 0.33	74.37± 0.45	76.03± 0.75	78.54± 0.37	-

CDR: cumulative drug release

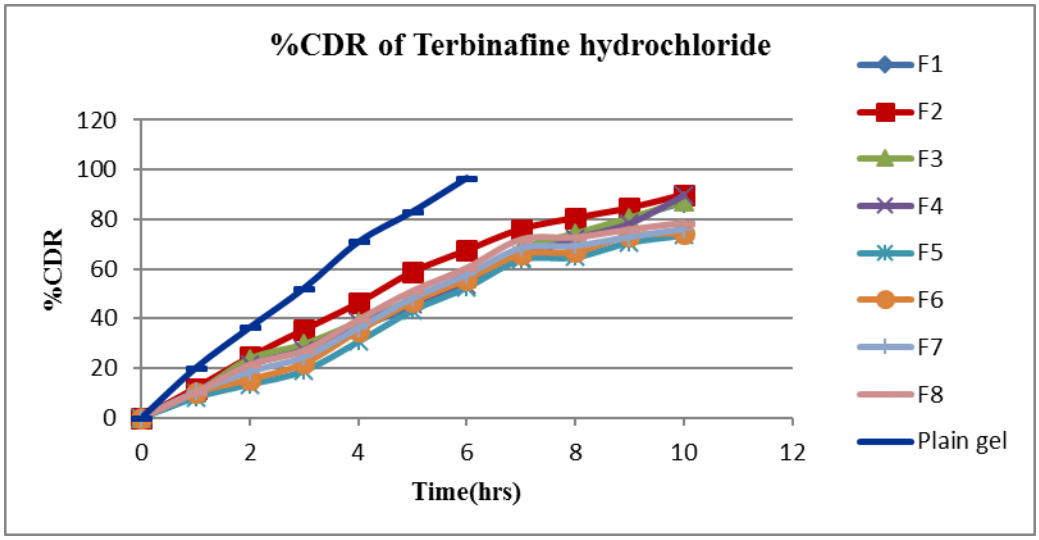

Figure 5: Comparison of percentage cumulative drug release profile of formulations F1-F8 and plain gel

From the *in-vitro* drug release data of Terbinafine hydrochloride microsponge based gel, it was observed that the percentage cumulative drug release of Terbinafine hydrochloride decreased as the concentration of ethyl cellulose was increased. The increase in the ethylcellulose concentration leads to the increased density of polymer matrix of microsponges which results in an increased diffusional pathlength. This may decrease the overall drug release from the polymer matrix. The optimum sustained release of drug was shown by formulation F2. F2 released 89.80% of the drug in 10h.

Minimum concentration of emulsifying agent is required to bring about the formation of uniform and stable microsponges. Insufficient concentration of emulsifying agent may produce unstable and non-uniform microsponges. This, in turn, reduces the drug release from the microsponges. F5-F8 contains microsponges formed using low concentration of emulsifying agent compared to F1-F4. Therefore, F5 to F8 showed a decrease in drug release compared to F1 to F4.

Terbinafine hydrochloride loaded plain gel showed a percentage cumulative drug release of 96.65 after 6hrs. From these results, it was evident that Terbinafine hydrochloride loaded microsponge gels sustained the release of drug when compared to drug loaded plain gels. The drug release of microsponge enriched gels showed a slower release when compared to plain gel formulation.

7. Kinetics of *in-vitro* drug release

The *in-vitro* drug release data of all the Terbinafine hydrochloride microsponge based gel formulations and drug loaded plain gel was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equations, Higuchi's and Korsmeyer–Peppas models to ascertain the mechanism of drug release.

Table 10: Kinetic study of microsponge based gel formulations and drug loaded plain gel

Formulation	Drug release kinetics				
	Zero order R^2	First order R^2	Higuchi R^2	Peppas	
				R^2	n
F1	0.994	0.954	0.932	0.989	0.958
F2	0.972	0.982	0.959	0.989	0.883
F3	0.993	0.955	0.941	0.992	0.915
F4	0.997	0.911	0.931	0.997	0.920
F5	0.976	0.976	0.907	0.979	1.048
F6	0.972	0.980	0.923	0.978	0.964
F7	0.969	0.979	0.934	0.985	0.921
F8	0.965	0.980	0.941	0.986	0.918
Drug loaded plain gel	0.995	0.855	0.940	0.998	0.886

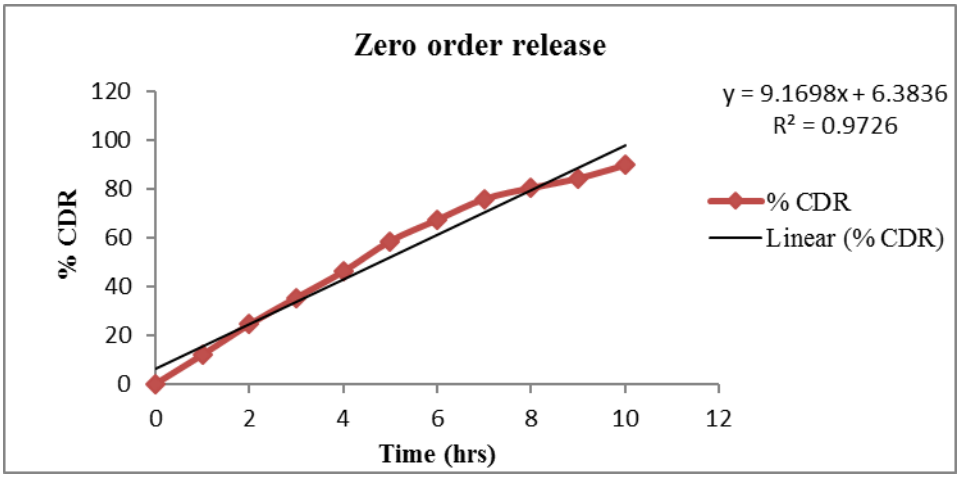

Figure 6: Zero order release kinetics profile of optimised formulation F2

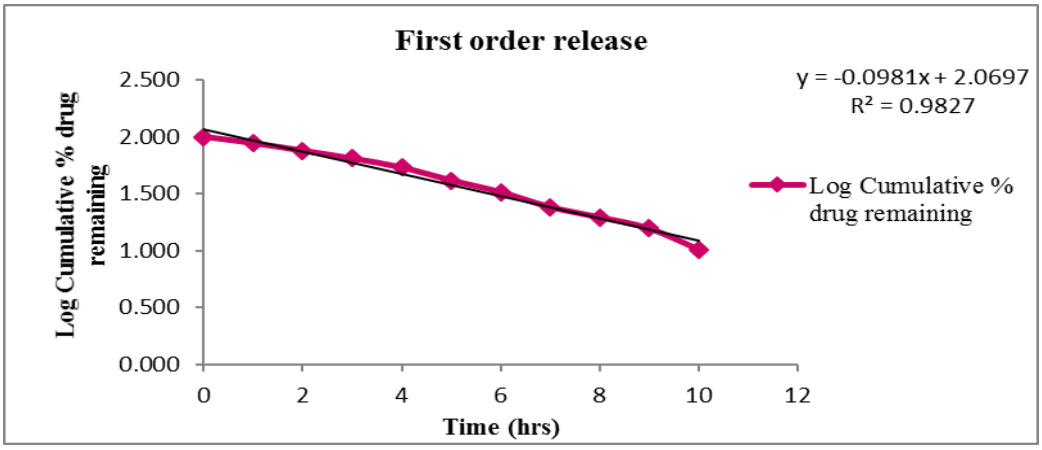

Figure 7: First order release kinetics profile of optimized formulation F2

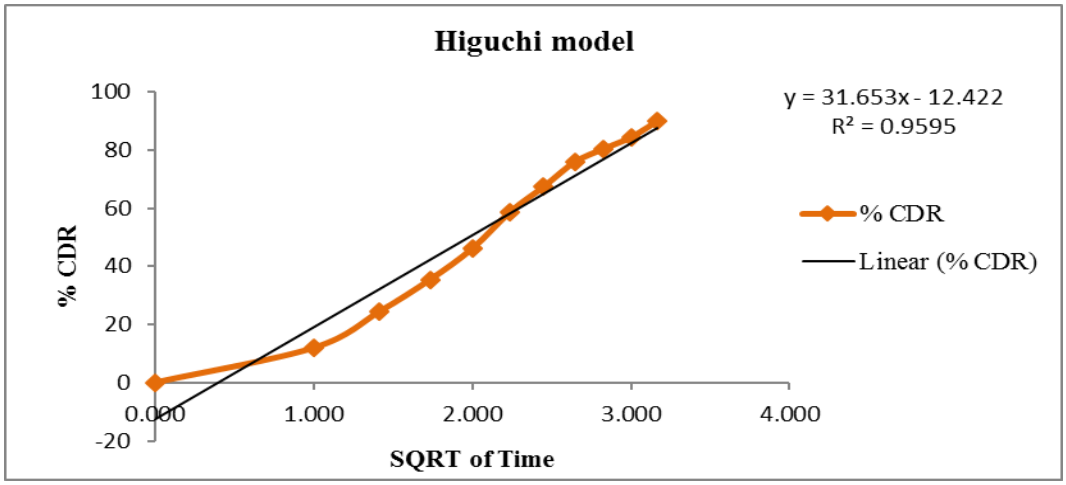

Figure 8: Higuchi release kinetics profile of optimized formulation F2

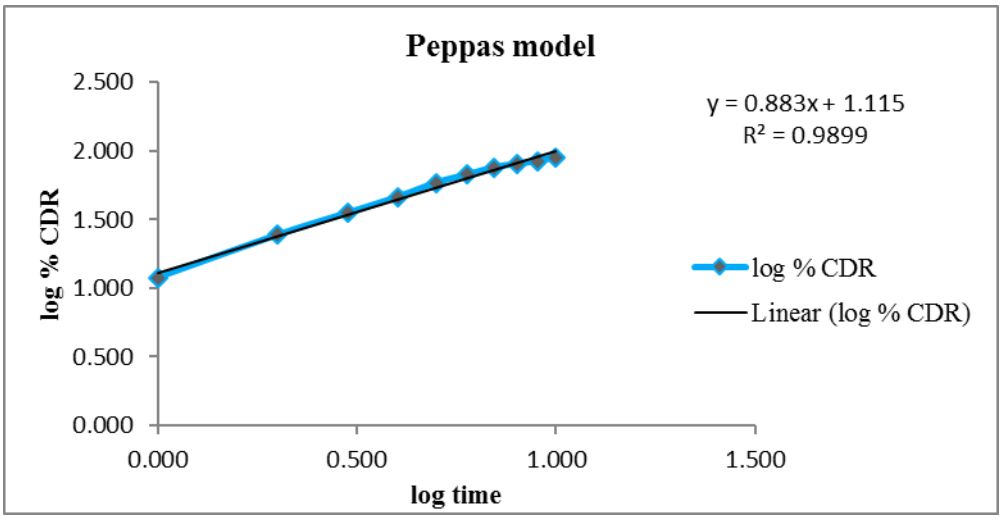

Figure 9: Peppas release kinetics profile of optimized formulation F2

From the above data, it was found that formulations F1, F3, F4 and drug loaded plain gel follows zero order kinetics with R^2 values 0.994, 0.993, 0.997 and 0.995 respectively. While, the formulations F2, F6, F8 follows first order kinetics with R^2 values 0.982, 0.980 and 0.980 respectively. To ascertain the drug release mechanism, the *in-vitro* drug release data were also subjected to Korsmeyer-Peppas plot. The 'n' values of optimized microsponge gel formulation F2 (n=0.883) and drug loaded plain gel (n=0.886) suggests that the drug was released by first order kinetics with non-Fickian (anomalous) release. Formulation F5 showed super case II release with 'n' value 1.048. All other formulations followed case II non-Fickian mechanism.

8. Rheological studies

The rheological properties of the optimized formulation of Terbinafine hydrochloride loaded microsponge based gel (F2) were determined using Rheometer MCR51 by frequency sweep analysis.

Table 11: Rheological data obtained from frequency sweep analysis

Log Angular frequency (rad/s)	Log Storage modulus (Pa)	Log Loss modulus (Pa)	Log Complex Viscosity (Pa.s)
2	3.269513	2.980458	1.320146
1.800029	3.276462	2.932474	1.515874
1.599883	3.262451	2.883661	1.698101
1.399674	3.217484	2.836324	1.85248
1.198657	3.227887	2.808886	2.056905
1	3.209515	2.776701	2.238046
0.800029	3.1959	2.748188	2.419956
0.599883	3.176091	2.729165	2.60206
0.399674	3.158362	2.70927	2.78533
0.198657	3.146128	2.702431	2.97174
0	3.130334	2.696356	3.158362

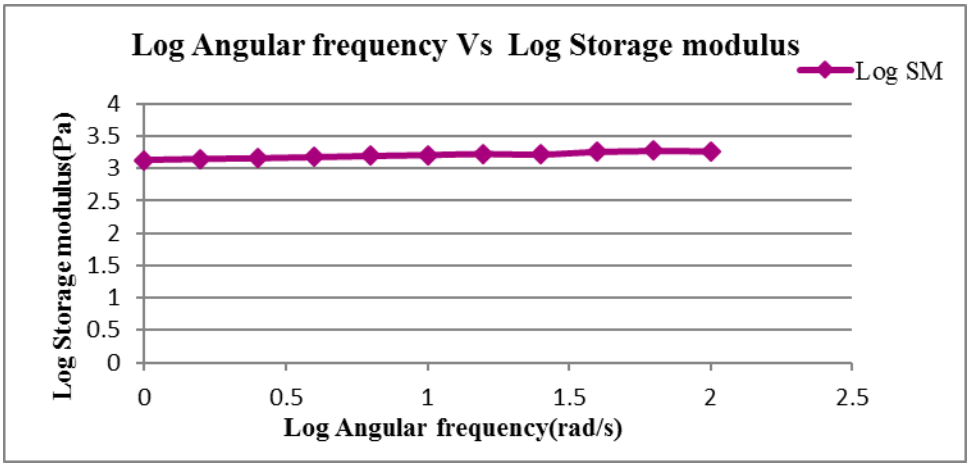

Figure 10: Graphical representation of Log Angular frequency Vs Log Storage modulus

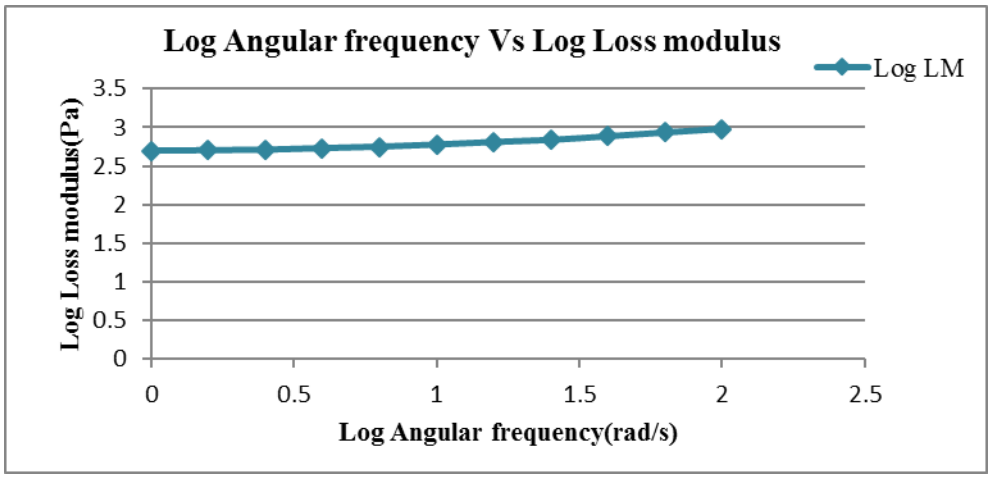

Figure 11: Graphical representation of Log Angular frequency Vs Log Loss modulus

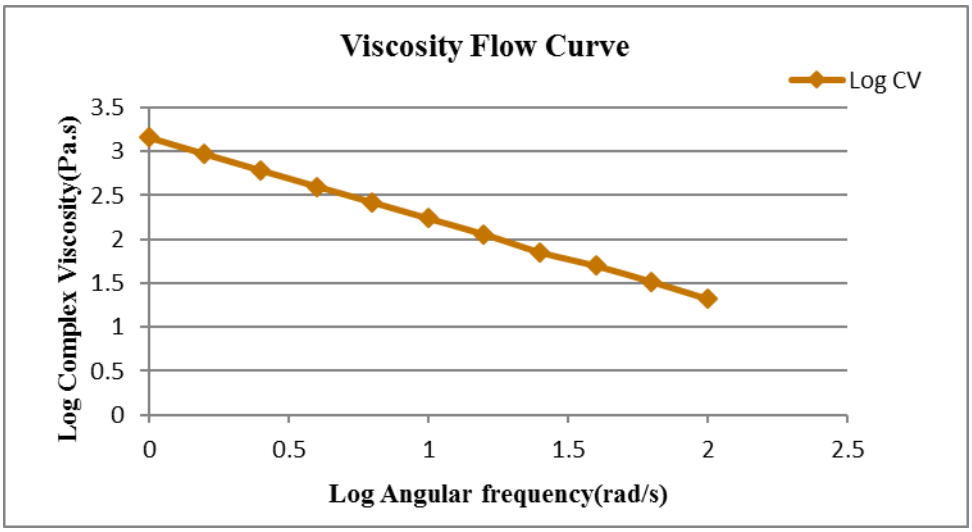

Figure 12: Viscosity flow curve of optmized formulation F2

Figure 10 indicated that, when the angular frequency was increased, the storage modulus also increased. Storage modulus is an indication of the gel's ability to store deformation energy in an elastic manner. From the **Figure 11**, it was found that the loss modulus increased with increasing angular frequency. Loss modulus is the measure of energy dissipated due to the viscous flow.

From the viscosity flow curve of optimized microsponge based gel formulation F2 shown in **Figure 12,** it was found that the formulation follows non-Newtonian flow indicating decrease in complex viscosity at increasing angular frequency (shear rate). This decreased viscosity of the formulation, due to the increasing shear rates indicated that the formulation shows shear-thinning behavior or pseudo-plasticity. It could be generally concluded that the visco-elastic nature of the microsponge based gel was good indicator of its stability.

9. Evaluation of antifungal activity by disk diffusion method

The optimized formulation showed clear zone of inhibition around the sample disc and it is shown in **Figure13.**

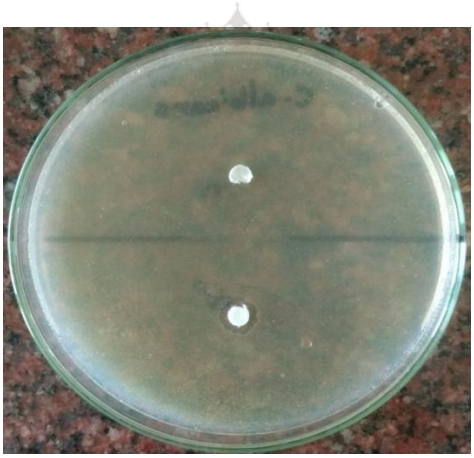

Figure 13: Clear zone of inhibition showed by formulation F2

10. Stability studies

Stability studies were carried out on optimized formulation F2 for a period of one month. The comparison of the parameters before and after stability studies were represented in **Table 12.**

Table 12: Comparison of parameters before and after stability studies

Parameters	Before stability studies	After stability studies
Appearance	white colored microsponges suspended in transparent gel base	white colored microsponges suspended in transparent gel base
pH	5.8	5.6
Drug content (%)	91.12	90.54
%CDR (10 hrs)	89.80	88.98

The results obtained from the stability studies showed that the optimized formulation F2 showed only a slight decrease in the drug content of Terbinafine hydrochloride at 40^{o}C after 1 month of storage. The *in-vitro* drug release also slightly decreased after the stability period. This may due to the decrease in the relative drug content. There was no change in the appearance of the formulation and observed only a slight difference in pH. From the stability studies, it was confirmed that the optimized formulation of Terbinafine hydrochloride microsponge based gel remained stable at 40°C and 75% relative humidity.

CONCLUSION

Terbinafine hydrochloride loaded microsponges were successfully prepared by using quasi-emulsion solvent diffusion method. All of the prepared Terbinafine hydrochloride loaded microsponges were evaluated for physical properties, particle size, surface morphology, production yield and drug entrapment. The prepared microsponge based gel formulations were consistent, viscous, smooth and homogenous and appeared like white coloured microsponges suspended in transparent gel base and they were evaluated for pH, drug content, skin irritancy, spreadability, *in-vitro* release, rheological properties, antifungal activity and stability. The optimum sustained release of drug around a period of 10h was shown by formulation F2. The 'n' value from Peppas model for the optimized formulation F2 and drug loaded plain gel indicated that the drug release follows anomalous (non-Fickian) release. From the rheological studies, it was concluded that the optimized formulation F2 shows shear-thinning behaviour or pseudo-plasticity. Based on the above evaluation studies, it was concluded that the formulation F2 was considered as optimized formulation and it was

safe and effective for topical use as Terbinafine hydrochloride loaded microsponge based gel and showed sustained release effect with reduced side effects.

ACKNOWLEDGEMENT

We are extremely grateful to Mar Dioscorus College of Pharmacy, Thiruvananthapuram, Kerala for the facilities provided to complete this work successfully.

REFERENCES

1. DebjithBhowmik et al. Recent advances in novel topical drug delivery system. The Pharma Innovation. 2012; 1(10) : 24-30.
2. Ghanshyam Das Gupta et al. Recent advances in topical drug delivery systems. Indo American Journal of Pharmaceutical Research. 2016; 6(7) : 6353-6355.
3. Leon Shargel, Susanna Wu-Pong, Andrew Yu. Chapter 17- Modified release drug products. Applied Biopharmaceutics and Pharmacokinetics. 6th edition, 2012; 469-476.
4. KatkadeMayur et al. Ethylcellulose based microsponge delivery system for anti-fungal vaginal gels of tioconazole. Journal of Drug Delivery and Therapeutics. 2013;3(6) :14-20.
5. E. K. Patel, R.J. Oswal. Nanosponge and microsponges-a novel drug delivery system. International Journal of Research in Pharmacy and Chemistry. 2012; 2(2): 237-242.
6. AvhadPawan.S, PatilPrashant.B. A new era in topical formulations-Microsponge drug delivery system. International Journal of Pharmaceutical Sciences and Research. 2016; 7(7): 2756-2761.
7. BasavaiahKanakapura, Vamsi Krishna Penmatsa. Analytical methods for determination of terbinafine hydrochloride in pharmaceuticals and biological materials. Journal of Pharmaceutical Analysis. 2016; 6(1): 137-149.
8. S.Dineshmohan, V.R.M.Gupta. Formulation and in-vitro evaluation of fluconazole loaded microsponge gel for topical sustained delivery. Journal of Pharmacy and Biological Sciences. 2015; 10(6): 15-20.
9. TusharShetgaonkar, R.NarayanaCharyulu. Microsponge drug delivery of terbinafine hydrochloride for topical application. Int. J. Pharm. Sci. Rev. Res. 2015; 33(1):48-54.
10. Kokane Vikrant, NaikSonali. Formulation and evaluation of topical flurbiprofen gel using different gelling agents. World Journal of Pharmacy and Pharmaceutical Sciences. 2014; 3(9): 654-663.
11. Marwa.H..Shukr, Ghada.F.Metwally. Evaluation of topical gel bases formulated with various essential oils for antibacterial activity against methicillin resistant Staphylococcus aureus. Tropical Journal of Pharmaceutical Research. 2013; 12(6): 877-884.
12. Maria.L.Demartine, E.L.Cussler. Predicting subjective spreadability, viscosity and stickness. Journal of Pharmaceutical Sciences. 1975; 64(6): 976-982.
13. Dinesh Kumar Shah, Amit Agrawal. Formulation and evaluation of polymeric microsponges of ketoconazole for topical delivery. World Journal of Pharmacy and Pharmaceutical Sciences. 2016; 5(10): 939-962.
14. Suvakanta Dash et al. Kinetic modeling on drug release from controlled drug delivery systems. ActaPoloniaePharmaceutica-Drug Research. 2010; 67(3): 217-223.
15. David J Mastropietro et al. Rheology in Pharmaceutical Formulations-A Perspective. Journal of Developing Drugs. 2013; 2(2): 1-6.
16. V.Varalakshmi, R.Mala. Effect of herbal extract on antimicrobial susceptibility profile of drug resistant burn wound isolates. International Journal of Agriculture, Environment and Biotechnology. 2013; 6: 815-821.
17. Kim Huynh-Ba. Chapter 1-Introduction. Handbook of stability testing in Pharmaceutical development. 1st edition,2011: 1-2.