ejpmr, 2017,4(06), 326-335

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FORMULATION AND EVALUATION OF TOLNAFTATE MICROSPONGES LOADED GELS FOR TREATMENT OF DERMATOPHYTOSIS

Pushpa Kumari[1], Shashi Kiran Misra*[2] and Shraddha Pandey[1]

[1]University Institute of Pharmacy, C.S.J.M.U, Kanpur.
[2]Assistant Professor, University Institute of Pharmacy, C.S.J.M.U, Kanpur.

***Corresponding Author: Shashi Kiran Misra**

Assistant Professor, University Institute of Pharmacy, C.S.J.M.U, Kanpur.

Article Received on 14/04/2017 **Article Revised on 05/05/2017** **Article Accepted on 26/05/2017**

ABSTRACT

Dermatophytosis is caused by dermatophytes (fungi) that can invade the epithelial tissue or stratum corneum. It extensively occurs in warm humid environment and in immuno-compromised persons. Dermatophytes are classified into three genera, Trichophyton (M.canis, M.gypseum), Microsporum (M.canis, M.gypseum) and Epidermophyton (E.floccosum) which causes dermatophytosis such as tinea pedis, tinea corporis, tinea mannum, tinea cruris and tinea capatis. Here we formulated Tolnaftate loaded topically active microsponges by quassi emulsion solvent diffusion method for effectively eradicating dermatophytes. Two grades of Eudragit (ERL 100 and ERS 100) alone and in combination were selected to evaluate for their active role in controlled drug release at target site. The microsponges were characterized through and evaluated for FTIR, SEM, DSC, TGA studies, particle size, drug content, in vitro drug release, following peppas release kinetics and selected for formulation of topical gels and anti fungal activity. From in vitro drug release study it was concluded that microsponge formulated by ERL 100: ERS 100 (3:1) exhibited maximum controlled release & this formulation was subjected for topical active gels preparation. On applying various drug release kinetics model it was found that formulated gels followed peppas model with diffusion coefficient less than 0.5, describing fickian diffusion type of drug release. Hence formulated tolnaftate loaded gels have potential to treat dermatophytosis and can be used to treat other tinea infections.

KEYWORDS: Microsponges, Dermatophytosis, Tolnaftate, ERL/ERS 100, Tinea pedis.

INTRODUCTION

Dermatophytes are among the most common eukaryotic pathogens dermatophytosis caused by filiform keratinophilic fungi that use human & animal keratin as a nutrient for infection.[1] Fungal infections have become important public health concerns. The fungal infections are the most opportunistic infections in the immuno-compromised individuals and warm humid environment causing various illnesses. Dermatophytes are classified into three genera, *Trichophyton (T.rubrum, T.mentagrophyte), Microsporum (M.canis, M.gypseum) & Epidermophyton (E.floccosum).* Their infections are termed as tinea pedis, tinea corporis, tinea mannum, tinea cruris and tinea capitis based on the infection site. These *Microsporum* and *Trichophyton* are human and animal causative whereas *Epidermophyton* is a human microorganism.[2] Dermatophytes demand keratin for their growth, they are resides on hair, nails and superficial skin. Therefore, it can be treated with topical antifungal drug.[3,4,5] Dermatophytes are occupied in the stratum corneum interior the keratinocytes. The signs and symptoms that arise in influence characteristics are due to acute and chronic inflammatory variation that develop in the dermis.[6,7] The vast superiority of antifungals are fungistatic with the concentrations attained in the skin when applied topically the growth of dermatophytes is retarded and these are exfoliate with the skin regenerated.[8,9]

Tolnaftate is a synthetic thiocarbamate used as antifungal drug. It discovered by Noguchi and colleagues in Japan in 1962[10] has been abundantly used as a manner of topical antifungal in the therapeutics of dermatophytosis such as Jock itch, athlete's foot and other skin infections.[11,12] Tolnaftate is topically used in a concentration of 1% in creams, lotions, powders, solutions, aerosols, gels to treat various fungal infection of tinea pedis (affects the foot), tinea cruris (jock itch) affects the groin area, tinea corporis (affects the arms, legs & trunk), tinea capitis (affects the scalp), tinea manuum (affects the hands and palm area)and tinea nigra caused by Hortaea werneckii.[13,14] Tolnaftate inhibits squalene epoxidase which essential for formation of ergosterol required for cell wall of fungi.[15,16] The accumulation of squalene and deficiency of ergosterol

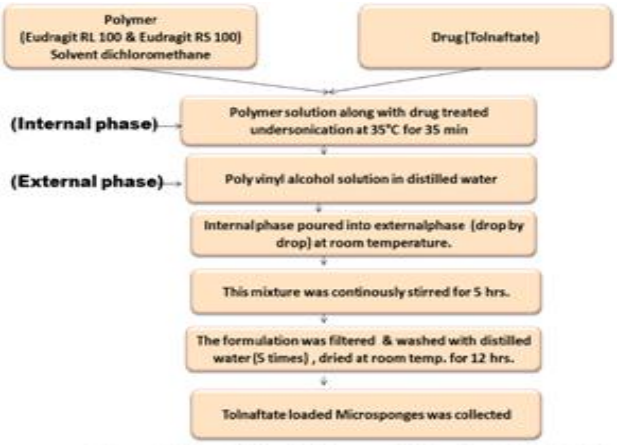
results in antifungal action of tolnaftate.[17,18] Two different grade of polymer used in microsponges i.e, eudragit RL 100 & eudragit RS 100 both are bio-compatible in nature & eudragit RS 100 also have anti-taking properties. Eudragit RS 100 is less permeable as compared to eudragit RL 100 due to its present lesser content of quaternary ammonium groups.[19,20] Novel approache to increase efficiency of the drug. Microsponges drug delivery discussed in this paper to eradicate the growth of *TM (Trichophyton mentagrophytes)* with controlled drug release and effective fungicidal property thereby without composed the adequancy is through colloidal drug delivery system. Colloidal or sub-micron drug delivery systems are designing application in the pharmaceutical research for the advertising as they increasing the permeability, raised the drug efficacy, slower release makes constant and prolonged action and very low essential toxicity.[21,22,23] Microsponges are porous microspheres having myriad of interconnected voids of particle size ranging between 5-300 µm.[24,25,26] Microsponges consist of non-collapsible structures with porous surface through which active ingredients are released in controlled manner.[27,28] Microsponges are stable over range of pH 1 to 11 and temperature up to 130˚C, are compatible with most vehicles and ingredients and self sterilizing as their average pore size is 0.25µm where bacteria cannot penetrate These formulations are free flowing and can be cost effective.[29,30] The release of drug into the skin can be initiated by a various triggers like rubbing, concentration gradient, higher skin temperature, application of pressure).The Microsponge system can prevent excessive accumulation of ingredients within the epidermis and the dermis. Thus, can significantly reduce the irritation of effective drugs without reducing their efficacy.[31]

MATERIAL AND METHOD

Tolnaftate was obtained from Yarrow chem products, Mumbai. Eudragit RL 100, Eudragit RS 100, Carbopol 934 and HPMC were purchased from Yarrow chem products, Mumbai. Poly vinyl alcohol, poly ethylene glycol, dichloromethane, methnol and triethanolamine were purchased from S.D. Fine Chemicals Ltd., Mumbai. All other reagents and chemicals used were of analytical reagent grade.

Preparation of microsponges

Tolnaftate microspnges were prepared by quassi emulsion solvent diffusion method using an internal phase, consisting of Eudragit RL 100 (ERL), Eudragit RS 100 (ERS) alone and in their combinations of ratio (1:1, 1:3, 3:1); Each dissolved in 5ml dichloromethane, followed by addition of PEG (Poly ethylene glycol 400) of 0.5ml was added to enhance the plasticity. There after tolnaftate 250mg added and allowed for ultrasonication at 35ºC. External phase was consisting of PVA (Poly vinyl alcohol) in 10ml distilled water at 60. The external phase was allowed to cool to attain room temperature. The internal phase was poured into external phase drop wise with dropper at continuously strirring 6-7 hrs at 500 rpm shown in (Figure 1). The microsponges were formed due to the removal of dichloromethane from the system by evaporation, washed with distilled water 4-5 times, filtered and dried at room temperature for 12 hour.[32,33,34] Various formulation of tolnaftate loaded microsponges along with their variables are shown in (Table 1).

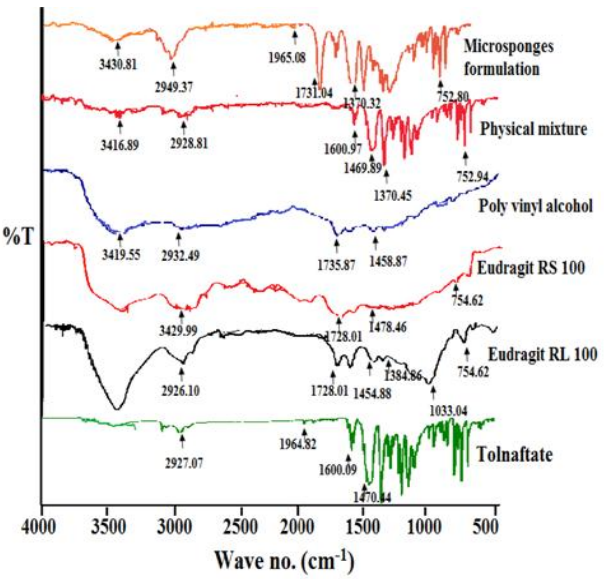


Quassi Emulsion Solvent Diffusion Method

Characterization

Fourier transform infrared (FTIR) analysis

Identification and chemical interactions were studied by FTIR spectroscopy. Tolnafate, polymers and physical mixtures of samples were individually mixed properly with potassium bromide to a uniform mixture. A small quantity of the powder was compressed into a thin semi-transparent pellet by applying pressure. The IR spectrum of the samples from 500-4000cm^{-1} was recorded taking as the reference and compared.[35]



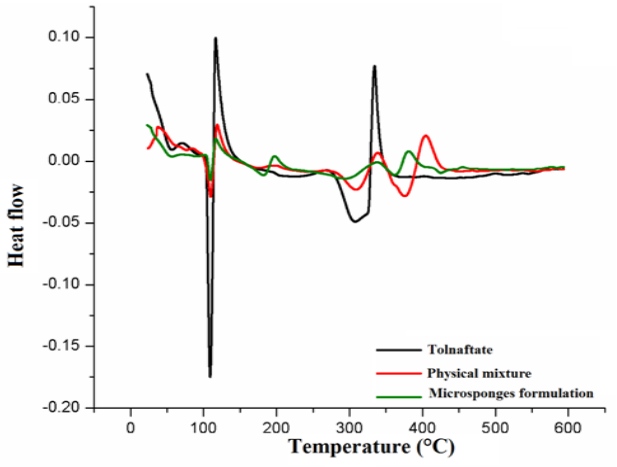
Differential scanning calorimetry (DSC) analysis

Differential scanning calorimetry (DSC) was carried out to determine the compatibility between the drug and excipients (polymer & surfactant) and also used to evaluate the crystalline state of drug, polymer, physical

mixture and formulation by using (Shimadzu DSC-60 Thermal Analyzer). Accurately weighed samples (5mg) were loaded into aluminum pans and sealed. All samples were run at a heating rate of 10ºC/min over a temperature rang 50-600ºC in atmosphere of nitrogen.[36]

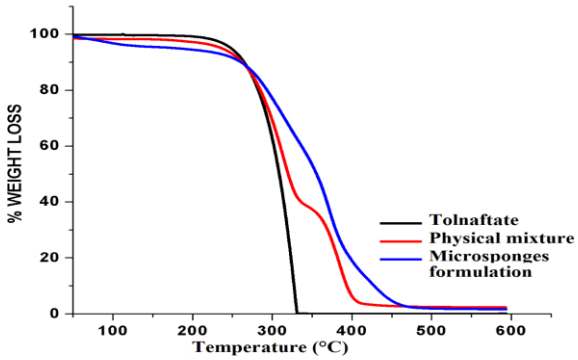
Thermal gravimetric analysis (TGA)

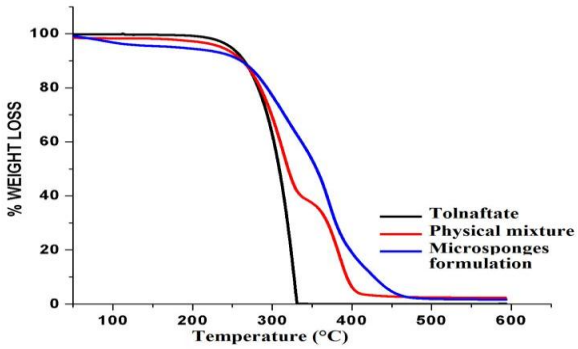
TGA recorded the decrease in the weight of the sample as a function of the temperature range 20ºC-400ºC. TGA indicated the dehydration and decomposition of the each tolnaftate, physical mixture and microsponges.[37]



Particle size determination

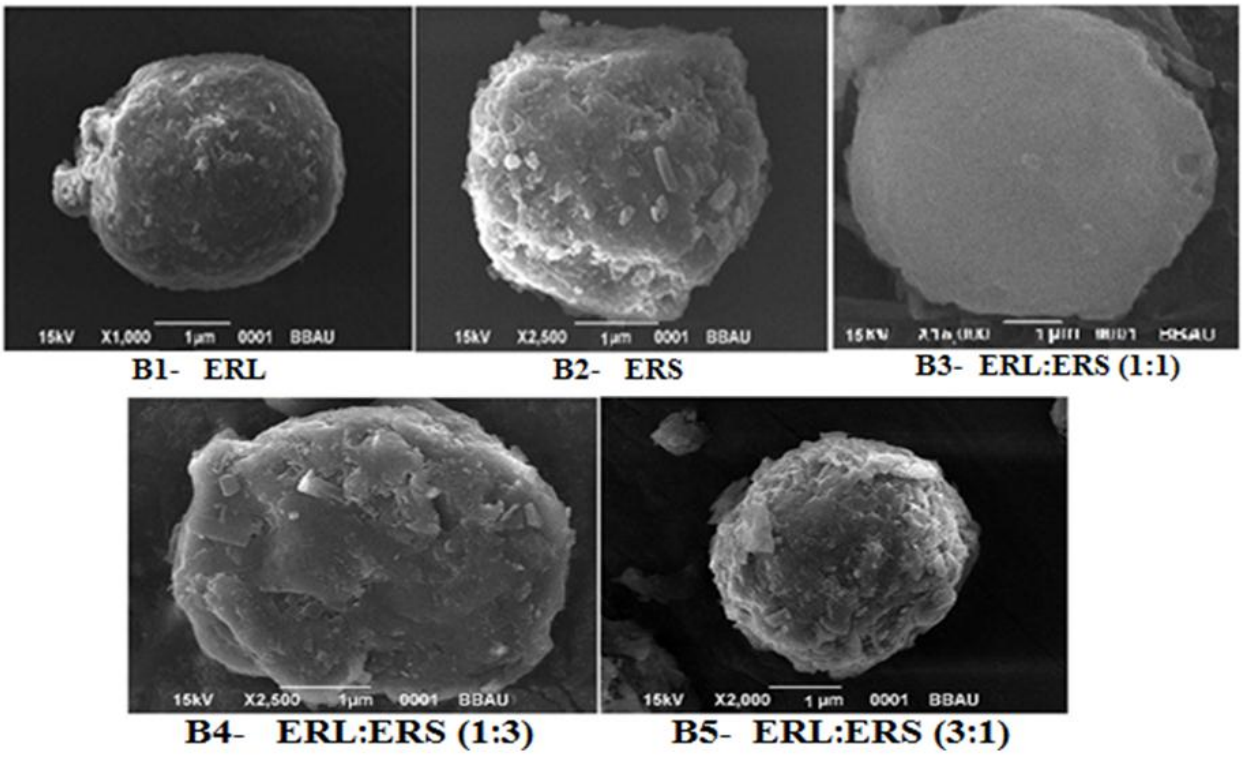
The particle size was determined using both simple & optical microscope (Olympus). At least 100 particles of microsponges were counted for precise size distribution.[38]





Scanning electron microscope (SEM) analysis

For morphology and surface topography examination of the dried tolnaftate loaded microsponges was carried out using a scanning electron microscope equipped with secondary electron detector at an accelerating voltage of 10 kV. The samples were coated with gold to a thickness of about 30 nm in a vacuum evaporator.[39]

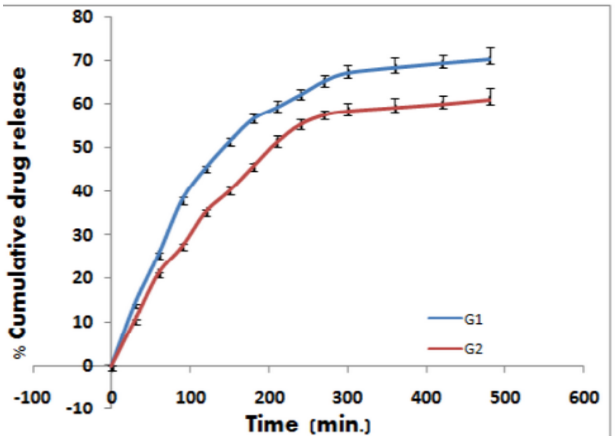


Determination of production yield

The production yield of Tolnaftate loaded microsponges of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microsponges, percent production yields were calculated as per the formula mentioned below. Percentage yield = Practical yield/ Theoretical yield×100.[40]

Determination of drug content

A sample of dried 10mg tolnaftate microsponges was taken in to mortar and pestle and added little amount of phosphate buffer pH 6.8 and allowed to stand for 24 hours. Then transfered content in to 100 ml volumetric flask and made up volume to 100 ml with phosphate buffer pH 6.8 and was filtered through whatman filter paper. Drug content was determined by UV spectrophotometer (Shimadzes, 1700) at 257 nm.[41]



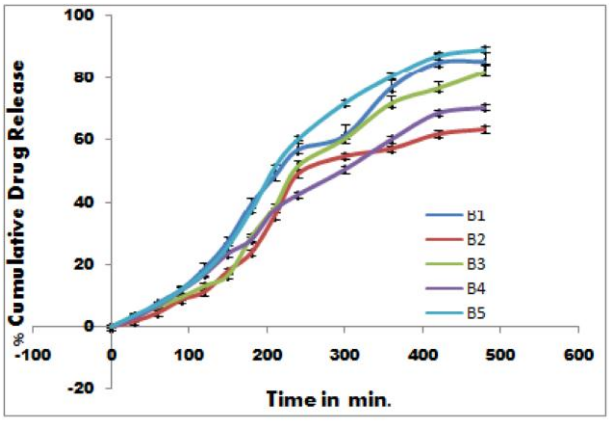
In vitro release by dialysis bag method

Accurately weighed microsponges equivalent to 10mg tolnaftate drug was kept in dialysis bag along with 5ml phosphate buffer pH 6.8. Tied dialysis bag was kept in beaker containing phosphate buffer pH 6.8 and allowed for diffusion for 8hrs. At predetermined time intervals of 30, 60, 90, 120, 150, 180, 240, 300, 360 and 420 min, 5mL aliquots were sampled and replaced with 5 mL fresh phosphate buffer pH 6.8, which was maintained at 37 ± 0.5 °C. In order to maintain sink conditions, an equal volume of medium was replaced. The samples were analyzed by the UV-visible spectrophotometer at 257 nm and drug release profile was estimated.[42]

Formulation of microsponges entrapped gels

Batch B5 was reported better as it exhibited maximum controlled drug release and it is selected to formulate topical gel. For formulation of gels, two polymer carbopol and HPMC were selected. Equal amount of concentration both polymer was choose to compare their gelling properties. Dissolved accurately weighed quantity of carbolpol 934 and HPMC in 20ml distilled water to this added solvent blend comprising of ethanol:propylene glycol which is previously contained tolnaftate loaded microsponges with constant stirring and also added propyl paraben was added for preservative. To the whole mixture, drop wise triethanolamine was added until transparent gel was obtained. Heavy stirring was avoided to escape entrapped air, further formed gel was stored in an air tight collapsible tubes for further study.[43,44,45] The formulation variables were given in (Table II).

Evaluation of Microsponges loaded gel



Appearance

The physical appearance through visual inspection confirmed that both microsponges loaded gels were successfully formulated.[46,47]

Determination of pH

The pH of the gel was measured using pH meter by putting the tips of the electrode in to the gel and after 2 minutes the result was recorded.[48]

Spreadability

The spreadability of all gel formulation was determined by using horizontal plate method lg of gel was placed between two horizontal glass plates and standard weight 125 g was placed on upper glass plate . The time was noted for the gel from one plate to side off from the other plate. The spreadability was calculated by using the following formula:

$$S = M \times L / T$$

Value "S" is spreadability, m is the weight tied to the upper slides, "l" is the length of glass slide, and "t" is the time taken.[49]

Determination of Extrudability

The formulation was filled in the collapsible tube after the gel was set in the container. The extrudability of the formulation was determined in terms of weights in grams required to extrude a 0.5 cm ribbon of gel in 10 seconds.[50]

Viscosity measurement

Viscosity of both gels formulation was determined using Brookfield viscometer with spindle No. 63 at 1.5 rpm at temperature 37±0.5°C.[51]

Drug content studies

100 mg of each gel formulations were taken in 100 ml volumetric flask containing 100 ml of phosphate buffer pH 6.8 and stirred for 30 minutes and allowed to stand for 24 hours in case of microsponges loaded gel formulations. The volume was made up to 100ml and 1ml of the above solution was further diluted to 50ml with phosphate buffer pH 6.8. The resultant solution was filtered through membrane filter of 0.45µm. The absorbance of the solution was measured through UV spectrophotometer at 257 nm.[53]

***In vitro* drug release by Dialysis bag method**

Accurately weighed microsponges loaded gels equivalent to 10mg tolnaftate was kept in dialysis bag and subjected for dialysis for diffusion of drug for 8hrs. At predetermined time intervals of 30, 60, 90, 120, 150, 180, 240, 270, 300, 330, 360, 390, 420 min, 5ml aliquots were sampled and replaced with 5 mL fresh methanolic phosphate buffer pH 6.8. The samples were analyzed by the UV-Visible spectrophotometer at 257 nm to determine the concentration of drug release.[53]

In vitro anti-fungal activity by Agar Diffusion Method

In vitro antifungal activity was carried out by agar diffusion method. 250 ml of PDA media, were taken in sterile conical flask inoculated 0.5ml of suspension of the *TM* (*T.mentagrophyte*) micro-organism. The PDA suspension of micro- organism was mixed well with loop. The media of fungi was prepared 250ml. These media were dispersed in sterile petriplates and allowed to solidified. After solidified the media hole were made by with sterile cork borer in such way that the well are separated by not less not 20 mm from each other and 10mm edges of the plate. By mean 5ml of sterile dropper, about 50mg of formulated topical sample individually (pure drug, control microsponges, control gel, drug loaded microsponges, microsponges loaded gels) was added to each well and also their control. All petriplates were incubated for 37°C for 24 hrs area of zone of inhibition was observed.[54]

RESULTS AND DISCUSSION

Fourier transform infrared (FTIR) analysis

FTIR spectra were recorded to assess the compatibility of the drug and excipients (Figure 2). FTIR spectra of drug, Eudragit RL 100, Eudragit RS 100, physical mixture of drug, polymer (Eudragit RL 100, Eudragit RS 100) & polyvinyl alcohol (PVA) and formulated microsponges were examined. All characterstics peaks of tolnaftate, PVA, eudragit RL100 and eudragit RS 100 were observed in the FTIR spectra of microsponges. The results showed that no chemical interaction or change took place during formulation of microsponges and drug was found to be stable. FTIR spectra of Tolnaftate, characteristic peak of C-N carbamate stretching at1275.44cm^{-1}, C=S stretching at 1964.82cm^{-1}, C=C stretching at 1627.14cm^{-1} were obtained. FTIR spectra of PVA revealed observed C-CH_2 bending alkane stretching at 1458.87cm^{-1}, N-C=O carbonyl stretching alkane vibration on amides at 1735.87cm^{-1}, C-H bending vinyl stretching at 1375cm^{-1} and C-H stretching in CH_2 at 2932.49cm^{-1}. FTIR spectra of eudragit RL 100 and eudragit RS 100 were exhibited O-H stretching of hydrate band at 3429.99cm^{-1}, C=O stretching at saturated aldehyde at 1735.65cm^{-1}, N-R stretching of quaternary amine salt at 1384.86cm^{-1} and C-CO-C stretching of strong band of ester at 1735.65cm^{-1}. All characterstics peaks of tolnaftate, PVA, eudragit RL100 and eudragit RS 100 were observed in the FTIR spectra of microsponges. The results showed that no chemical interaction or changes took place during formulation of microsponges and drug was found to be stable.

Differencial scanning calorimetry (DSC) analysis

DSC studies were carried out to confirm drug polymer compatibility (Figure 3). The thermal behavior of drug, physical mixture and microsponges were studied out which summarized endothermic peak of tolnaftate at 111ºC, corresponding to the melting point of drug in the crystalline form. Melting point at 111ºC revealed peak was also appeared in DSC of physical mixture and formulated microsponges.

Thermal gravimetric analysis (TGA) studied

The TGA curve indicated slight decrease in total weight of the tolnaftate around at 110ºC due to melting of drug tolnaftate. After that it showed 10% weight loss at 340ºC attributed to decomposition. Physical mixture showed significant weight loss as temperature increase upto (temp.) attributed to presence of ERL 100, ERS 100, microsponges and PVA (poly vinyl alcohol). TGA of formulated microsponges described stability for upto 350ºC indicating its better thermal behavior (Figure 4).

Particle Size analysis

The formulation of microsponges batches B1 to B5 were prepared by using drug, eudragit ER 100, eudragit RL 100 and ratio of eudragit RL 100:eudragit RS 100 (1:1, 1:3, &3:1). Microsponges were found to almost slightly spherical and showed some irregularity in its structure. Microsponges B1 exhibited regular surface as compared to B2 may be attributed to chemical compostion of ERS 100. Formulations B3, B4, B5 even almost regular and spherical paticle. The particle size distribution of microsponges formulation of batches B1, B2, B3, B4 & B5 showed an average particle size of 0.4µm, 0.2µm, 0.6µm, 0.8µm and 1.0µm respectively. The particles size distributions of tolnaftate microsponges were depicted in (Figure 5a & Figure 5b).

Scanning electron microscopy (SEM) analysis

The morphology of the microsponges formulated were investigated by Scanning electron microscopy. SEM images of the microsponges are shown in (Figure 6a & Figure 6b) was found that almost all batches have spherical porous surface it was seen that formulation B3 equal concentration of eudragit RL 100 and eudragit RS 100 so some porosity on their surface.

Determination of production yield

Production yield determined for all batches B1 to B5 microsponges ranged from 67.6, 63.4, 68.8, 68.9 & 71.7%. From the production yields of tolnaftate microsponges formulation, it was indicated that in B5 higher production yield (Table.III).

Drug content studies

Drug content was determined through UV spectrophotometer and the sequence of drug present in all batches were 39.3(B5), 37.8(B3), 33.3(B1), 28.88(B2) & 27.3(B4) mg drug/100mg of microsponges reported in (Table III).

***In vitro* diffusion studies by dialysis bag method**

It was observed that the release rate of drug from Eudragit RL100 microsponges was a little higher than that of Eudragit RS 100 microsponges because Eudragit RL100 contains higher amount of quaternary ammonium groups, which renders it more permeable and accelerates the drug release. The release rate of Eudragit RS100 microsponges exhibit a lag time at the initial release and the best release was observed with formulation B5. Drug release of didderent batches of microsponges formulation were able release upto (63.4 to 89.1%) drug after 6 hrs. shown in (Figure 7). The results of in vitro dissolution studies of tolnaftate loaded microsponges formulations at phosphate buffer pH 6.8 shown in (Table IV). On applying various drug release kinetics model (zero order, first order, higuchi and korsermeyer-peppas) it was found that microsponges formulations batch B1 to B2 followed peppas model with diffusion coefficient less than 0.5, describing fickian diffusion type of drug release shown in (Table V).

Evaluation of Microsponges loaded gels

Appearance

The formulated gel prepared by carbopol as gelling agent was white in colour whereas gel formulated through HPMC was translucent (Table VI).

Determination of pH

The pH values of G1 (microsponges loaded carbopol 934 gel) and G2 (microsponges loaded HPMC K100M) recorded between 6.7 to 6.9 which lie in the normal pH range of the skin shown in (Table VI).

Spreadibility

The values of spreadability indicated that the gel was easily spreadable by small amount of shear. The spreadability of G1 (microsponges loaded carbopol gels) was 18.33 g.cm/sec while micrsponges loaded HPMC gels containing as gelling agent was 24.8 g.cm/sec, indicating spreadability of gel containing microsponges loade HPMC gels was high as compared to the microsponges loaded carbopol gels shown in (Figure VI).

Viscosity

Viscosity is an important parameter for characterizing the gels as it affects the mechanical and physical properties such as spreadability, consistency of the formulation which provide ease behavior of application on the skin surface and patient compliance drug at target site as well as release of drug. The comparative viscosity estimated through Brookfield viscometer of G1 & G2 is reported in (Table VI).

Extrudiblity

An ideal gel should possess optimum extrudibility on application to provide patient compliance. G1gel (microsponges loaded carbopol gel) exhibited more extrudibility as compare to G2 (microsponges loaded HPMC gel) may be attributed to its less viscosity which facilitated more extrusion from tube with in specified time (Table VI).

Drug content studies

Tolnaftate loaded microsponges content in the optimized microsponges loaded carbopol gel formulations was found to be 29.50 mg drug in 100 mg gels and microsponges loaded HPMC gel was found 15.2 mg drug in 100 mg gel. Higher drug content in carbopol gel as compare to HPMC gel may be attributed shown in (Table VI).

***In vitro* drug release study by dialysis bag method**

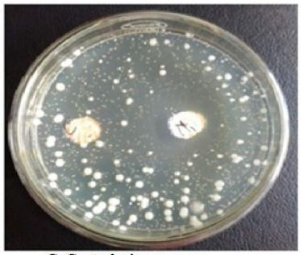
The drug release behavior study from topical gel indicated that the release of the drug was influenced by type and chemical nature of polymers. Very highly viscous solution and formed H-bond with drug molecule which reduced diffusion capacity. It was observed that maximum drug release from microsponge was achieved within 7 h. The diffusion study showed that permeation of drug containing G1 gels showed good permeability as compared to G2 gels (Figure 8). Both gels (G1 & G2) contain ethanol & propylene glycol which in combination enhances permeability of gel. Microsponges loaded carbopol gels shows higher drug release as compare to microsponges loaded HPMC gels result showed in (Table VII). On applying various drug release kinetics model (zero order, first order, higuchi and korsermeyer-peppas) it was found that formulated gels followed peppas model with diffusion coefficient less than 0.5, describing fickian diffusion type of drug release reported in (Table VIII).

***In vitro* antifungal activity using agar well diffusion method**

In vitro anti-fungal study revealed clear zone of inhibition and confirmed susceptibility of formulated microsponges and their gels towards dermatophyte T. mentagrophytes. Althrough formulated gels G1& G2 showed enhanced activity as compared to microsponges M1 may be attributed to more diffusion of drug from the gel to the petriplate (Figure 9a & Figure 9b) & (Table IX).

C2- Control HPMC gels
G2- Microsponges loaded HPMC gels

M1- Microsponges
G1- Microsponges loaded carbopol gels
G2- Microsponges loaded HPMC gels



C- Control microsponges
M1- Microsponges

C1- Control carbopol gels
G1-Microsponges loaded carbopol gels

Table 1: Different batches of tolnaftate loaded microsponges batch B1 to B5.

Formulation code	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Tolnaftate (mg)	250	250	250	250	250
Eudragit RL 100 (mg)	200	-	100	50	150
Eudragit RS100 (mg)	-	200	100	150	50
Dichloromethane (ml)	5	5	5	5	5
Poly vinyl alcohol (mg)	80	80	80	80	80
Plasticizer (ml)	0.5	0.5	0.5	0.5	0.5
Distilled water (ml)	10	10	10	10	10

Table 2: List of ingredient and their quantities of microsponges loaded gels.

S. No.	Ingredients	Formulation BF1 BF2	
1.	Microsponges (mg)	Equivalent to 100 mg drug	Equivalent to 100 mg drug
2.	Carbopol 934 (mg)	300 mg	-
3.	HPMC (mg)	-	300 mg
4.	Ethanol (ml)	3 ml	3 ml
5.	Propylene glycol (ml)	2 ml	2 ml
6.	Triethanolamine (ml)	2 ml	2 ml
7.	Methyl paraben (mg)	0.8 mg	0.8 mg
8.	Distilled water (ml)	50 ml	50 ml

Table 3: Comparative studies of production yield (%) & drug content.

Formulation code	Production yield (%)	Drug content (mg drug/ 100mg microponges)
Batch 1	67.6	33.3
Batch 2	63.4	28.88
Batch 3	68.8	37.8
Batch 4	68.9	27.3
Batch 5	71.7	39.3

Table 4: Cumulative drug release of microsponges formulations in phosphate buffer pH 6.8.

Time (min.)	Formulations of Tolnaftate loaded microsponges (% Cumulative Drug Release)				
	B1	B2	B3	B4	B5
0	0	0	0	0	0
30	3.1	1.8	3.5	2.9	3.7
60	7.6	4.5	6.4	6.8	7.2
90	11.9	8.6	9.4	11.6	11.8
120	18.6	11.2	13.5	16.8	17.4
150	27.2	17.8	16.4	23.4	25.7
180	39.4	23.6	28.6	27.8	37.4
210	48.3	35.4	38.4	37.6	51.3
240	56.8	49.2	51.6	42.1	60.2
300	61.2	54.8	60.2	50.5	71.8
360	76.6	57.2	71.6	60.2	80.4
420	84.8	61.9	76.4	68.3	87.5
480	85.3	63.4	81.7	70.4	89.1

Table 5: Release mechanisms of Tolnaftate loaded microsponges loaded batches B1 to B5.

S. No.	Formulation of tolnaftate loaded microsponges	Model				
		Zero order	First order	Higuchi	Peppas	n- value
		Coefficient correlation (r^2)	Coefficient correlation (r^2)	Coefficient correlation (r^2)	Coefficient correlation (r^2)	
1.	B1	0.9684	0.9264	0.9527	0.9689	0.890
2.	B2	0.9306	0.9168	0.9333	0.9307	0.876
3.	B3	0.9582	0.8943	0.9320	0.9627	0.879
4.	B4	0.9841	0.9096	0.9568	0.9844	0.893
5.	B5	0.9577	0.9036	0.9654	0.9926	0.896

Table 6: Evaluation parameters of tolnaftate microsponges loaded gels.

S.No.	Evaluation Parameters	Observation G1	Observation G2
1,	Appearence	Milky White in colour	Translucent
3.	pH of gel	6.74	6.92
5.	Viscosity	6079cp	6059c p
6.	Spreadibility	18.33 gcm/sec	24.8 gcm/sec
7.	Extradability	0.97g/cm^2	1 .03g/cm^2
8.	Drug Content	29.50mg drug in 100mg gels	15.2mg drug in 100mg gels

Table 7: In vitro drug release studies of Microsponges loaded gels.

Time (min.)	Formulations of microsponges loaded gels (% Cumulative Drug Release)	
	G1	G2
0	0	0
30	14.3	10.6
60	25.6	21.3
90	38.1	27.5
120	45.4	35.4
150	51.4	40.2
180	56.6	45.8
210	59.2	51.3
240	62.1	55.4
270	65.2	57.6
300	67.1	58.5
360	68.3	59.5
420	69.3	60.1

Table 8: Release mechanisms of Tolnaftate microsponges loaded gel of G1& G2.

S.No.	Model	Tolnaftate microsponges loaded gel					
		G1			G2		
		Correlation Coefficient (r^2)	Rate constant (k)	n-value	Correlation Coefficient (r^2)	Rate constant (k)	n-value
1.	Zero	0.878	0.205	-	0.8994	5.75	-
2.	First	0.8967	0.004	-	0.8056	0.003	-
3.	Higuchi	0.9688	0.721	-	0.9461	3.174	-
4.	Peppas	0.9745	5.718	0.423	0.9736	3.582	0.478
5.	Hixson	0.8122	0.8122	-	3.582	0.001	-

Table 9: Comparisions of antifungal activity of micrsponges loaded gels & pure drug.

Dermatophyte	Formulation	Zone of inhibition diameter in mm
T.mentagrophytes	Control microsponges	-
T.mentagrophytes	Control carbopol gels	-
T.mentagrophytes	Control HPMC gels	-
T.mentagrophytes	Microsponges	10.2±0.5
T.mentagrophytes	Micrsopnges loaded carbopol gels	8.9±0.5
T.mentagrophytes	Micrsopnges loaded HPMC gels	6.1±0.7

CONCLUSION

Tolnaftate loaded biocompatible microsponges were formulated through Quassi emulsion solvent diffusion method. Formulated microsponges were found to have spherical and porus surface, facilitating high pay load and better drug release. FTIR spectra of miocrosponges confirmed presence of drug and polymer by predicting wavenumber of their respective functionals groups. DSC and TGA analysis showed better thermal behaviour with no drug polymer interaction. Batch 5 Microsponges prepared from 3:1 ratio of Eudragit RL 100: Eudragit RS 100 showed higher drug release attributed to more permeability of Eudragit RL 100. The kinetic data of the in vitro diffusion release of tolnaftate microsponges from gel was found to follow peppas model kinetic on their (r^2) values give higher results of G1 than G2. Also best fit was found to be Higuchi matrix. Anti fungal activity through agar diffusion method revealed bio activity of formulated microsponges and their gels against dermatophyte *T. mentagrophyte*. The formulated gels containing microsponges have potential for treatment of dermatophytosis.

REFERENCES

1. White TC, Oliver BG, Graser Y, Henn MR. Generating and testing molecular hypotheses in the dermatophytes. J of Am Acad Dermatol, 2008; 7: 123-132.
2. Weitzman and Summerbell RC. The Dermatophytes. Cli Microbio Review, 1995; 8(2): 240-259.
3. Leyden JL. Tinea pedis pathophysiology and treatment. J of Am Acad Dermatol, 1994; 31(3-2): S31-S33.
4. Maraki S, Nioti E, Mantadakis, Tselentis Y, A 7-year survey of dermatophytoses in Crete Greece. Amer J Drug Del, 2005; 2007; 50(6): 481-484.
5. Schwartz RA. Superficial fungal infections. Lancet, 2004; 364(944): 1173-1182.
6. Elewski BE, Hazen PG. The superficial mycoses and the dermatophytes. J of Am Acad Dermatol, 1989; 21: 655-673.
7. De Vroey C. Epidemiology of ringworm (dermatophytosis). Am J Sci, 1985; 4: 185-200.
8. Philpot CM. Geographic distribution of the dermatophytes. A review J of Hygenic, 1978; 80: 301-313.
9. Gupta AK, Sander DN, Shear NH. Antifungal agents an overview Part I. J Am Acad Dermatol, 1994; 30: 677-698.
10. Chem X, Jini X. New Pharmacology. People's Medical Publishing House, Beijing, 1985; 12: 146-152.
11. Delgado J, Remer W. Text book of organic medical and pharmaceutical chemistry. Ausolters Kluwor company, philadelpha, 1998; 10: 191-199.
12. Cho G, Peng X. Assay of tolnaftate in comparison on the pharmaceutical compound preparation. Laboratories of foods and drugs investingation report, 1985; 3: 47-52.
13. Smith EB, Dickson JE, Knox JM. Tolnaftate powder in prophylaxis of inea pedis. Southern Med J, 1974; 67: 776-783.
14. Gupta AK, Chow M, Daniel CR, Aly R. Treatment of tinea pedis. Dermatology Cli, 2003; 21: 431-439.
15. Ryder NS, Frank I, Dupont MC. Ergosterol biosynthesis by the thiocarbamate. Anti-microbio Agents Chemothe, 1986; 29: 858-865.
16. Iwata K, Yamashita T, Uehara H. Potent inhibitory effects of N-aryl S-alkylthiocarbamate. Antimicrobio Agents Chemothe, 1989; 33: 2118-2129.
17. Barrett BK, Dixon G. Ergosterol biosynthesis inhibition a target for antifungal agents. Acta Biochemical Poloniae, 1995; 42: 465-473.
18. Crawford F, Harris R, Williams HC. Topical treatments for fungal infections of the skin. British J Dermato, 2008; 159: 773-785.
19. Toma K, Olga K, Audrius M, Vitalis B. Assay of tolnaftate in human skin samples after *in vitro* penetration studies using high performance liquid chromatography. Acta Poloniae Pharmaceutica Drug Res, 2010; 67(4): 327-334.
20. Tong MM, Altman PM, Barnetson RS. Tea tree oil in the treatment of tinea pedis. Australas J Dermat, 1992; 33: 145-153.
21. Noguchi T, Kaji A, Igarashi Y, Shigematsu A, Taniguchi K. Department of transfusion medicine and immunohematology. Chemotherapy, 1962; 12: 259-267.
22. Weinstein MJ, Oden EM, Moss E. Sisomicin is an aminoglycoside antibiotic. Antimicrobio Agents Chemother, 1964; 10: 595-617.
23. Venkata ST, Senhil V, Ilindra SK, Rizwan BK, Subba R, Madhunapantula V. Curr Drug Del, 2014; 11(4): 72-852.
24. Chowdary KPR, Rao Y S. Mucoadhesive microspheres for Controlled Drug Delivery. Bio Pharmace Bull, 2004; 27: 1717-1724.
25. Namrata J, Patel V, Mungekar S, Karpe M, Kadam V. Microsponge delivery system: An updated review current status and future prospects. World J Pharm & Pharmace Sci, 2007; 2(6): 6463-6485.
26. Vyas LK, Tapar KK, Laddha BH, Lahoti AO, Nema RK. Formulation and development of anti-blemish preparations using microsponge technology. J Chem Pharma Res, 2010; 2(5): 562-571.
27. Embil K, Nacht S. The Microsponge Delivery System (MDS) a topical delivery system with reduced irritancy incorporating multiple triggering mechanisms for the release of actives. J Microencap, 1996; 13(5): 575-88.
28. Aritomi H, Yamada K, Honda H, Koshi M. Development of sustained release formulation of chlorpheniramine maleate using powder coated microsponges prepared by dry impact blending method. J Pharmac Sci & Tech, 1996; 56(1): 49-56.
29. D'souza JI, Masvekar RR, Pattekari PP, Pudi SR. Microsponges delivery of fluconazole for topical

application. Indo-Japanese Inter Confer Adv Pharmac Res & Tech, 2004; 456: 76-81.
30. Partibhan KG, Manivannan R, Krishnarajan D, Chandra S, Nidhin R. Microsponges role in Novel drug delivery. Inter J Pharma Res & Devlop, 2011; 3(4): 117-1258.
31. Wester RC, Patel R, Nacht S, Leydan J, Malendres J, Maibch H. Controlled release of benzoyl peroxide from a porous microsphere polymeric system can reduce topical irritancy. J Am Acad Dermatol. 1991;24: 720-726.
32. Kilicarslan M, Baykara T. The effect of drug & polymer ratio on the properties of the Verapamil hydrochloride loaded microspheres. Inter J Pharma, 2003; 252: 99-109.
33. Chadavar V, Shaji J. Microsponge delivery system. Curr Drug Deli, 2007; 4: 123-129.
34. Tansel C¸ Omog L, Tamer B. Preparation and in vitro evaluation of modified release ketoprofen Microsponges. Il Farmaco, 2003; 58: 101-106.
35. Vikas J, Ranjit S. Development and characterization of Eudragit Rs 100 loaded microsponges and Its colonic delivery using natural polysaccharides. Acta Poloniae Pharmaceu Drug Res, 2010; 67(4): 407-415.
36. Bodmeier R, Chen H. Preparation and characterization of microspheres containing the anti-inflammatory agents, indomethacin, ibuprofen and ketoprofen. J Cont Rel, 1989; 10: 167-175.
37. Varma MM, Begum SKR. Formulation, physiochemical evaluation and dissolution studies of carbamazepine solid dispersion. Inter J Pharmace Sci & Nanotechnol, 2012; 5(3): 1790-1807.
38. Valmik SK, Shalini R, Kanchan M, Shwini C, Eknath P. Microsponge. Comprehensive review of application. Inter J Pharmac Bio Sci, 2013; 3(1): 214-226.
39. D'Emanuele A, Dinarvand R. Preparation, characterisation and drug release from thermoresponsive microspheres. Inter J Pharmac, 1995; 118(2): 237-242.
40. Mohan KV, Veena NM, Manjula BP. Formulation and evaluation of microsponges for topical drug delivery of mupirocin. Inter J Pharm Techno Res, 2013; 5(3): 1434-1440.
41. Yan Gao, Fu-de C, Ying G, Lei Y, Yong SW, Li-na Z: Preparation of Roxithromycin polymeric microspheres by the emulsion solvent diffusion method for taste masking. Inter J Pharm, 2006; 318: 62-69.
42. Srilatha R, Aparna C, Srinivas P, Sadanandam M. Formulation, evaluation and characterization of glipizide nanoemulsion. Asi J Pharmaco Cli Res, 2013; 6(2): 66-71.
43. Cooper G. Dispensing for pharmaceutical students. Pitman medical and scientific publishing company LTD, 1987; 11: 214-222.
44. Farhan JA, Mohd AA, Zeenat IK, Roop KK, Mushir A. Development and in vitro evaluatiaon of an acid buffering bioadhesive vaginal gel for mixed vaginal infections. Acta Pharmaceutical, 2008; 58: 407-419.
45. El-Houssieny B, Hamouda HM. Formulation and evaluation of clotrimazole from pluronic F127 gels. Drug discovery and therapeutics, 2010; 4(1): 33-43.
46. Setty CM, Babubhai SR, Pathan IB. Development of valdecoxib topical gel effect of formulation variables on the release of valdecoxib. Inter J Pharm Pharmace Sci, 2010; 2(1): 70-73.
47. Penzes T, Blazso G, Aigner Z, Falkay G, Eros I. Topical absorption of piroxicam from organogels invitro & invivo correlations. International Journal of Pharmaceutics, 2005; 298: 47-54.
48. Thakur N, Pratibha B, Mahant S, Rao R. Formulation and characterization of Benzoyl Peroxide Gellified Emulsions. Scientia Pharmaceutica, 2012; 80: 1045-1060.
49. Joshi B, Singh G, Rana AC, Saini S. Development and Characterization of Clarithromycin Emulgel for topical delivery. Inter J of Drug Develop & Res, 2012; 4(3): 310-323.
50. Katkade M, Kalkotwar R, Jain N, Patil P, Gadakh R, Naikwade J. Ethyl cellulose based microsponge delivery system for anti-fungal vaginal gels of tioconazole. J Drug Deli Therap, 2013; 3(6): 14-20.
51. Harish NM, Prabhakara P, Subrahmanyam EV. Formulation and evaluation of in situ gels containing clotrimazole for oral candidiasis. Ind J Pharma Science, 2009; 71(4): 421-427.
52. Sekhara B, Reddy CH, Tirupati SR, Rao RK, Duppada DR, Sarubudeen M. Formulation and evaluation of topically applied acyclovir loaded microsponges for the treatment of viral skin disorders. Inter J Innovative Pharma Sci Res, 2014; 2(10): 2412-2424.
53. Shazly G, Nawroth T, Langguth P. Comparison of dialysis and dispersion methods for *in vitro* release determination of drugs from multilamellar Liposomes. Dissolution technologies, 2008; 5(2): 7-9.
54. Chaman S, Sharma G, Reshi KA. Study of antimicrobial properties of *Catharanthus roseus* by agar well diffusion method. Inter Res J Pharma Sci, 2013; 3(5): 65-68.