ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Formulation and Evaluation of Ketoconazole Microsponge Topical Gel

Joshna Booravilli*, Janaki Devi Sirisolla, Shivani Saluru

GITAM School of Pharmacy, GITAM (Deemed to be University), Rushikonda, Visakhapatnam – 530045, Andhra Pradesh, India

Article History:

Received on: 20 Sep 2022
Revised on: 21 Oct 2022
Accepted on: 22 Oct 2022

Keywords:

Ketoconazole,
Eudragit L 100,
Eudragit S 100,
Quasi Emulsion Method,
Topical Gel

ABSTRACT

Microsponges are those drug delivery systems which are intended in a way to deliver a minimum dose of the drug or pharmaceutically active ingredient to increase the stability of formulation, to alter drug release profile. Ketoconazole is an imidazole anti-fungal agent belonging to BCS class II. The main objective of this research work is to formulate and evaluate ketoconazole microsponge for topical delivery by using various polymers such eudragit S-100, eudragit L-100 in four ratios 1:2, 1:3, 1:4, 1:5 by quasi emulsion method. The formulations prepared were labelled based on the ratios as ES 2. ES 3, ES 4, ES 5 for eudragit S-100 and EL 2, EL 3, EL 4, EL 5 for eudragit L-100. Evaluation tests like entrapment efficiency, production yield, drug content was performed and formulation of eudragit L-100 (EL 5) and eudragit S-100 (ES 5) showed better results. ES 5 and EL 5 formulations were optimized and tests like *in vitro* dissolution studies were conducted which showed that the formulation ES 5 showed released upto 8 hours and can be used in the formulation of gel. The gel (GES5) was prepared by usingmicrosponges containing the drug ketoconazole equivalent to 1 % w/w and was incorporated into the gel base made up of Carbopol 934. The microsponges were sieved and were dispersed in Carbopol gel. Evaluation tests like pH measurement, visual inspection, spreadability studies, drug content and *in vitro* diffusion studies. This method of formulating microsponges has improved drug delivery therefore helping in the enhancement of the bioavailability of the drug and also acts as an efficient carrier through the skin.

*Corresponding Author

Name: Joshna Booravilli
Phone: 8341885668
Email: bjoshna43@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v13i4.3630

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INTRODUCTION

There have been increased investigation done on the novel drug delivery system in order to achieve controlled release of drugs at the desired target site. This is due to major disadvantage of the conventional drug delivery systems which require high dose of active agents to be incorporated for showing therapeutic response due to their decreased efficiency as delivery systems [1]. Microsponges are highly porous, cross-linked, polymeric microspheres that have the ability to entrap various active ingredients that are used mainly for prolonged release of the drug via topical administration and also for oral administration recently [2].

The transdermal/topical delivery route of administration has various advantages than other delivery systems which includes continuous delivery of the drug to the targeted site, reduced side effects and improved patient compliance and avoiding first pass metabolism. Topical products are used exter-

nally for localized action in different layers of the skin (e.g., Sunscreens, local anaesthetics, keratolytic agents, antiseptics).

The main objective behind formulation and designing of Microsponges are to deliver the drug efficiently at a low dose and also to increase stability, reduced side effects and to alter the drug release profile [3]. These drug delivery systems prevent excessive accumulation of formulation within the epidermis and the dermis. Due to their porous nature, these microsponges can entrap various active ingredients such as sunscreens, essential oils, fragrances, emollients and anti-infective, antifungal, and anti-inflammatory agents [4].

Gels are semisolid dosage forms in which the liquid phase is entrapped within a three-dimensional polymeric matrix made up of natural or synthetic gums by physical or chemical cross-linking.The increased viscosity and the semi-solid state is caused by the interlacing property. When the gelling agents are dispersed in suitable solvent, they combine or entangle to form a three-dimensional colloidal network structure. This network structure of gels provides resistance against deformation and hence shows viscoelastic properties. The elasticity of certain gels may be due to the presence of double helix structure, similar to a water uptake capacity and to the rheological profile of each polymer tested [5].

MATERIALS AND METHODS

Materials

Ketoconazole, Eudragit L 100 was received from Yarrow Chemicals Private limited-Mumbai (India), Eudragit S 100 belongs to research grade and was received from Yarrow Chemicals Private limited-Mumbai (India), Carbopol 934, Polyvinyl alcohol, Ethanol was received from Changshu Hong sheng Fine Chemical Co Ltd.

Equipment

Shimadzu UV spectrophotometer, Hicon tray dryer, Dissolution apparatus, Franz diffusion cell apparatus, Petri dish, Sieve 24.

Methods

Analytical Method

The analytical method used is the spectroscopic method which was performed by measuring the absorbance at 244nm in methanol solution which was used for the estimation of ketoconazole [6–8].

Preparation of Standard Solution

100 mg of accurately weighed ketoconazole was transferred into 100ml of volumetric flask and was dissolved using methanol and the volume was made upto 100ml with the methanol.

Construction of Standard Curve

The standard solution of ketoconazole was taken as 1m, 2ml, 3ml, 4ml, 5ml to obtain various dilutions containing 10,20,30,40,50 μg/ml solutions. The absorbance of these solutions of various concentrations was measured in Shimadzu UV-1800 (Japan) UV spectrophotometer at wavelength 244 nm by using methanol as blank.

Preparation of Ketoconazole Microsponges

1. Drug and polymer were accurately weighed according to the ratios and dissolved in 5ml of ethanol on a magnetic stirrer to form the internal phase.
2. Appropriate amount of PVA was weighed and dissolved in water using mechanical stirrer to form an external phase.
3. Then internal phase was then poured in a dropwise manner into the external phase by gradually stirring using a mechanical stirrer.
4. The formed microsponges as shown in Figure 1 were filtered using Whatman filter paper and dried at 40°c for 12 hours.

Figure 1: Prepared Ketoconazole Microsponges

Evaluation Tests

Drug Content Estimation

Determination of the actual drug content was done by weighing the amount of drug-loaded microsponges (100 mg) which was suspended in 100 ml phosphate buffer pH 7.4 with continuous stirring for 2 hours. These samples were then filtered by using a Whatman filter and then these samples were analysed against blank at 244 nm using a UV-spectrophotometer (Shimadzu, UV 1800) [9].

Encapsulation Efficiency (EE)

Encapsulation efficiency (EE) was determined by weighing required amount of microsponges containing drug (100 mg) and placing in 100 ml phosphate buffer of pH 7.4 for 2 hours with continuous

stirring. Then the samples were filtered and analysed against blank at 244 nm using a UV spectrophotometer (UV 1800, Shimadzu).

$$Entrapment \quad efficiency = \frac{Mass\ of\ drug\ entrapped\ in\ microsponge}{Initial\ mass\ of\ drug} \times 100$$

Percent Yield (PY)

For the determination of percent yield (PY), drug-loaded microsponges (100 mg) were weighed and was placed in 100ml of phosphate buffer pH 7.4 for 2 hours by continuous stirring. These samples were then filtered and analysed against blank on a UV spectrophotometer at 244 nm (UV 1800, Shimadzu).

$$Percent\ \ yield = (Mass\ of\ the\ microsponges\ obtained/ (Initial\ mass\ of\ the\ drug + Initial\ mass\ of\ polymers\ in\ the\ drug)) \times 100$$

***In Vitro* Dissolution Studies**

In vitro dissolution studies were done using a USP (type 1) apparatus i.e. basket type with 50 rpm at a temperature of 37±0.5°. Drug release studies were carried out initially in 900 ml of 0.1 N HCl for 2 hours then in phosphate buffer pH 6.8 for the next 4 hours followed by pH 7.4 phosphate buffer for another 6 hours. At regular intervals of time samples were withdrawn and each time were adjusted by adding equal volume of freshly prepared dissolution medium in order to maintain the sink conditions. These samples were then analysed at a wavelength of 244 nm spectrophotometrically.

Formulation of Microsponges Loaded Ketoconazole Gel

Microsponges prepared by using Quasi emulsion solvent diffusion method were weighed accurately equivalent to ketoconazole 1 % w/w was then combined into the gel base. Carbopol 934 was soaked in 10 ml of water overnight and dispersed by agitation at 600rpm using stirrer to form a gel base. The microsponges were sieved using sieve no 10 and were uniformly dispersed in Carbopol gel as shown in Figure 2. Triethanolamine was added as permeation enhancers.

Evaluation of Ketoconazole Gel

Visual Inspection

The gel formulation containing microsponges was visually inspected by their colour, appearance and texture [10].

pH Measurement

The pH of the prepared gel formulation was determined with the help of digital pH meter. It was done by taking one gram of gel which was dissolved in 100 ml distilled water and was then stored for two hours [11–13].

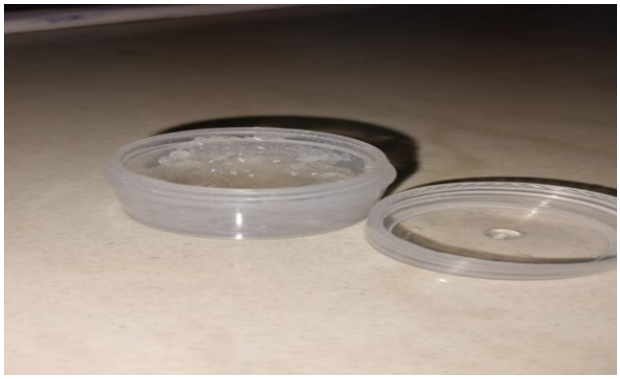

Figure 2: Preparation of Ketoconazole Gel

Spreadability Studies

Spreadability is the measurement of time in seconds taken by two glass slides to slip off from gel which was placed in between the glass slides by applying certain load. Less the time taken for the separation of two glass slides, better is the spreadability [11–13]. These studies were done as shown in Figure 3.

It was calculated using the following formula:

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

Where, S denotes the spreadability; M is the mass in the pan (which is tied to the upper slide); L indicates length moved by the glass slide; T is the time required to separate the two slides completely from each other.

Figure 3: Spreadability Studies Done Using Weighing Balance

Drug Content

Accurately weighed 1 gram of ketoconazole microsponge gel was taken in pH 7.4 phosphate buffer of 100 ml for 2 hours and was filtered. The absorbance was measured by UV spectrophotometer at 244 nm against blank gel treated similar to the sample (Table 1, Table 2).

***In Vitro* Diffusion Study**

In vitro diffusion studies of the gel were carried out across the semi-permeable dialysis membrane.

Phosphate buffer saline of pH 7.4 was filled inside the receptor compartments [14–16].

Franz diffusion cell which has an effective area 4.52 cm^2 was placed on a magnetic stirrer whose temperature can be kept at 37°C throughout the study as shown in Figure 4. The optimized formulations of drug loaded microsponge were used for the diffusion study. Samples each of 5 ml volume were collected at different time intervals and was replaced by equal volumes of the buffer solution in the receptor medium. Then the withdrawn samples were suitably diluted with the receptor medium. At regular intervals for about 12 hours release studies were carried out. Final release study samples were withdrawn and UV spectrophotometric studies were carried out at 244 nm [17, 18].

Figure 4: Franz Diffusion Cell Apparatus

RESULTS

Formulation of Ketoconazole Gel (GES5)

1. Different formulations of microsponges containing ketoconazole which are equivalent to 1 % w/w were induced into the gel base made up of Carbopol 934.
2. This Carbopol 934 was soaked overnight in 10 ml of water and was dispersed by agitation at 600 rpm using stirrer.
3. Before uniform dispersion of the microsponges in Carbopol gel, they were sieved.
4. Triethanolamine was used as permeation enhancers (Table 5, Table 6).

DISCUSSIONS

The calibration curve of ketoconazole was performed by using the values of absorbance vs concentration and a linear relation was found in the range of 0-50 μg/ml concentration. The R^2 value obtained was 0.997 as mentioned in Figure 4. Hence it obeyed

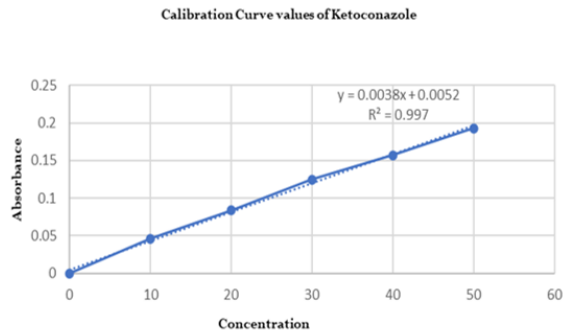

Figure 5: Calibration Curve of Ketoconazole at λ max 244nm

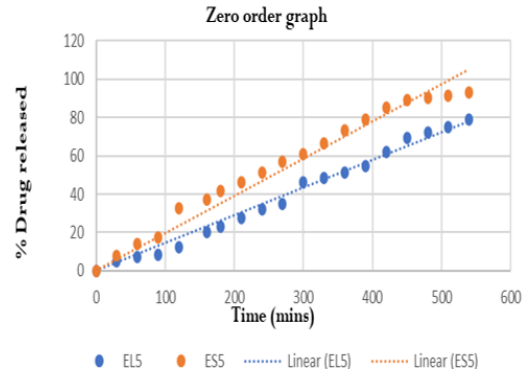

Figure 6: Zero Order Graph of Ketoconazole Microspheres EL5 (1:5) and ES5 (1:5)

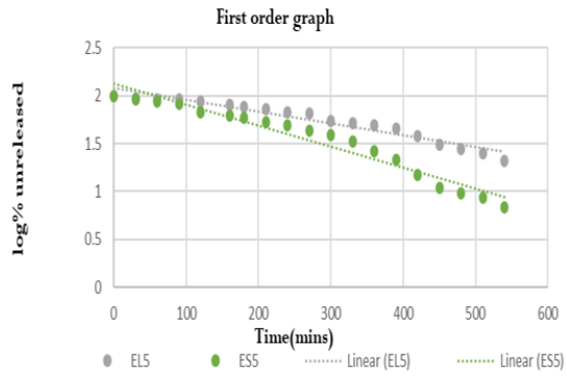

Figure 7: First-Order Graph of Optimized Microsponge Batches of EL5 and ES5

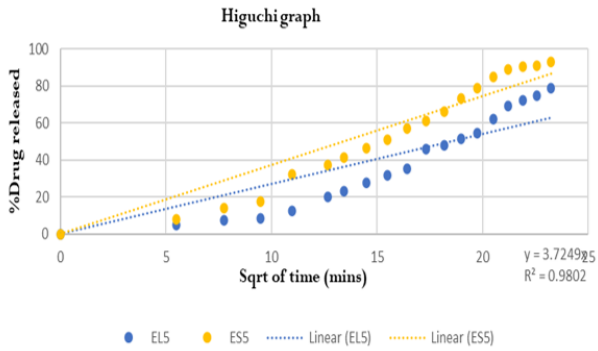

Figure 8: Higuchi Graph of Optimized Microsponge Batches of EL5 and ES5

Table 1: Standard Calibration Curve Values of the Drug Ketoconazole

S No	Concentration (mg/ml)	Absorbance (nm)
1	0	0
2	10	0.046
3	20	0.084
4	30	0.125
5	40	0.157
6	50	0.193

Table 2: Formulation of Ketoconazole Microsponges

	Eudragit L-100					Eudragit S-100				
	1:1	1:2	1:3	1:4	1:5	1:1	1:2	1:3	1:4	1:5
Chemicals	EL 1	EL 2	EL 3	EL 4	EL 5	ES 1	ES 2	ES 3	ES 4	ES 5
Polymer	100	200	300	400	500	100	200	300	400	500
Ketoconazole	100	100	100	100	100	100	100	100	100	100
Polyvinyl Alcohol	50	50	50	50	50	50	50	50	50	50
Ethanol (ml)	5	5	5	5	5	5	5	5	5	5

Table 3: Evaluation Parameters of Drug Loaded Microsponges

S No	Formulations	Production Yield (%)	Drug content (%)	Entrapment efficiency (%)
1	EL2	8.01%	9.01%	12.03%
2	EL3	20.21%	17.4%	50.7%
3	EL4	96.15%	45.5%	59.07%
4	EL5	98.04%	80.72%	77.61%
5	ES2	6.15%	8.22%	11.11%
6	ES3	10.56%	15.41%	49.7%
7	ES4	72.04%	36.53%	54.68%
8	ES5	92.12%	61.51%	71.03%

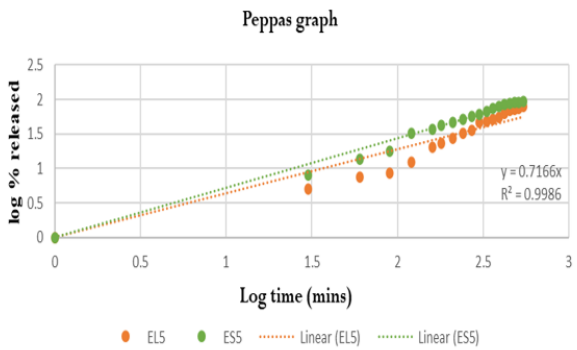

Figure 9: Peppas Graph of Optimized Microsponge Batches of EL5 and ES5

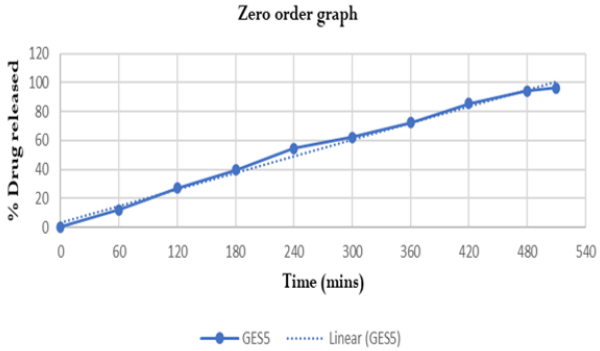

Figure 10: Zero-Order Graph of Ketoconazole Gel (GES5)

Beer Lambert's law (Figure 5, Figure 6, Figure 7, Figure 8, Figure 9, Figure 10, Figure 11, Figure 12 and Figure 13).

Evaluation Parameters of the Microsponges

Drug content, production yield and entrapment effi-

Table 4: *In Vitro* Dissolution of Optimised Formulations of EL 5 and ES 5

Dissolution Media	Time (mins)	Mean Percent (mean ± Sd)	Drug Release (n=3)
		EL 5	ES 5
	0	0	0
	30	4.96 ± 0.02	7.99 ± 0.02
pH 1.2	60	7.56 ± 0.01	13.82 ± 0.03
	90	8.64 ± 0.02	17.71 ± 0.01
	120	12.52 ± 0.03	32.4 ± 0.03
	160	20.01 ± 0.02	37.15 ± 0.02
	180	23.1±0.03	41.6 ±0.01
	210	27.62±0.01	46.4±0.02
pH 6.8	240	31.96 ± 0.02	51.19± 0.01
	270	35.20 ± 0.04	57.02± 0.03
	300	46.04 ± 0.01	60.91± 0.02
	330	48.20 ± 0.02	66.31± 0.01
	360	51.4 ± 0.01	73.41± 0.01
	390	54.5 ± 0.03	78.62± 0.02
	420	62.11 ± 0.02	85.10± 0.03
	450	69.13 ± 0.04	89.12± 0.02
pH 7.4	480	72.31± 0.03	90.31±0.01
	510	75.04±0.01	91.23±0.03
	540	79.1 ± 0.02	93.10± 0.01

Table 5: Kinetics of Drug Release from Ketoconazole Microsponges

Formulation	Zero order		First order		Higuchi	Peppas	
	R^2	K_0	R^2	K_1	R^2	R^2	N
EL5	0.990	3.063	0.945	2.07	0.943	0.905	0.614
ES5	0.996	6.136	0.974	2.12	0.980	0.982	0.716

Table 6: Evaluation of Ketoconazole Microsponge Gel (GES5)

Formulation	Visual Inspection	pH	Drug Content	Spreadability Studies
GES5	Translucent, White homogenous gel	7.1 ± 0.34	3.33±1.615	4.57±0.04

Table 7: *In-Vitro* Diffusion Release Studies Ketoconazole Gel (GES5)

Time (Mins)	% Drug Release
0	0
60	11.88±0.02
120	27.0±0.01
180	39.4±0.01
240	54.36±0.02
300	62.28±0.03
360	72.1±0.03
420	85.32±0.02
480	94.34±0.02
510	96.21±0.03

Table 8: Kinetics of Drug Release of Ketoconazole Gel (GES5)

Formulatio	Zero order		First order		Higuchi	Peppas	
	R^2	K_0	R^2	K_1	R^2	R^2	N
GES5	0.932	2.835	0.992	2.168	0.972	0.976	0.814

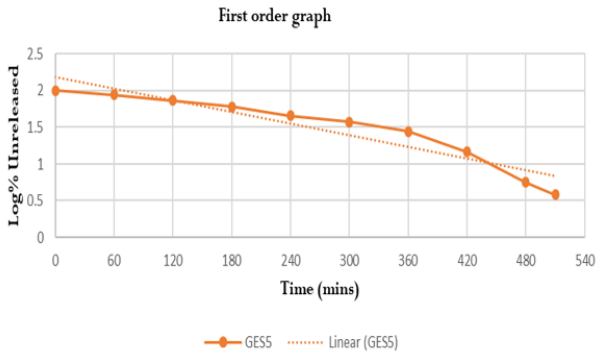

Figure 11: First-Order Graph of Ketoconazole Gel (GES5)

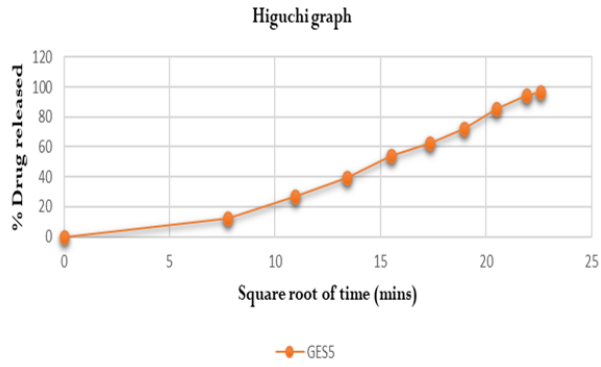

Figure 12: Higuchi Graph of Ketoconazole Gel (GES5)

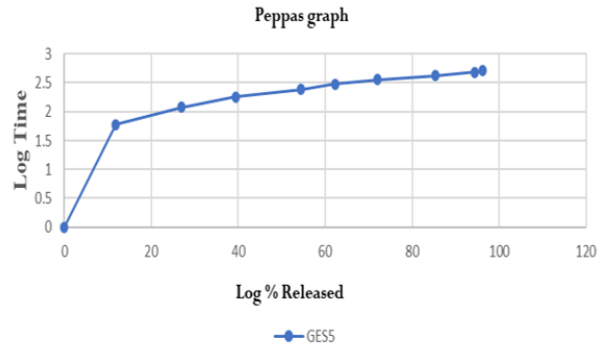

Figure 13: Peppas Graph of Ketoconazole Gel (GES5)

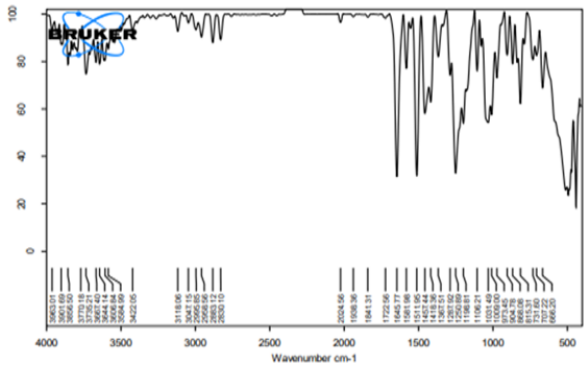

Figure 14: FTIR Spectra of Ketoconazole

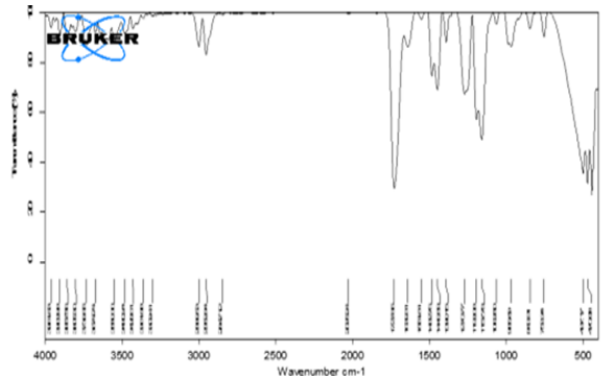

Figure 15: FTIR Spectra of Eudragit S-100 Polymer

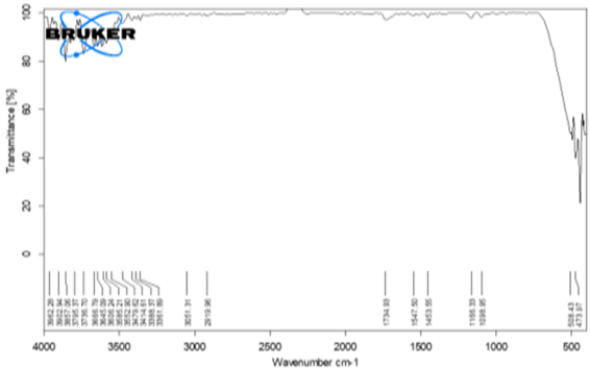

Figure 16: FTIR Spectra of Carbopol 934

ciency of different batches of microsponges were evaluated. Production yield ranged between 8.01 % - 98.04% for formulation with Eudragit L100, 6.15 % to 92.12 % for formulations with Eudragit S100 as mentioned in Table 3.

Entrapment efficiency for the formulation with Eudragit L-100 shows maximum upto 77.6%, for

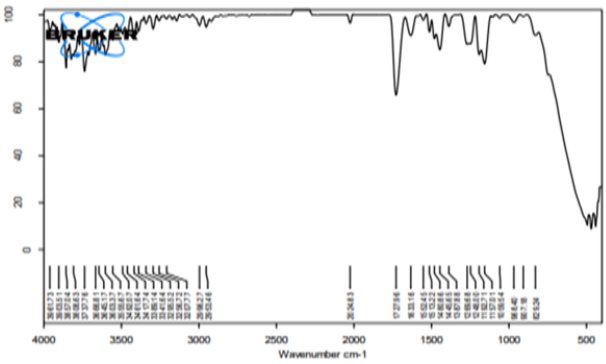

Figure 17: FTIR Spectra of Microsponges Eudragit L100

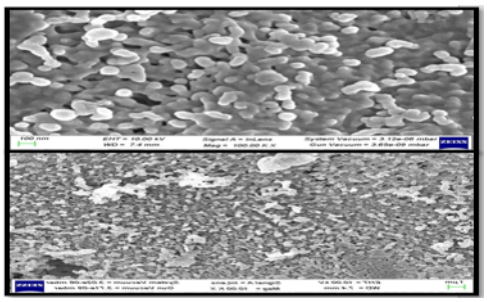

Figure 18: SEM Images of Microsponges

Eudragit S-100 shows 71.03%. Maximum amount of drug content was obtained in the formula with Eudragit L100 80.72%. Drug content for the formulation with Eudragit S-100 was 61.51%. Based on the above evaluation parameters results, formulation EL5 and ES5 were selected for dissolution studies.

Dissolution Studies and Kinetics of Drug Released from Ketoconazole Microsponges

The *In vitro* dissolution profile of the optimized formulations of EL 5 and ES 5 was performed. Initial 2 hours of dissolution in 0.1N HcL buffer showed that EL5 released up to 12.52% and ES 5 released up to 32.4% as shown in Table 4. The next 4 hours of dissolution was performed in phosphate buffer with pH 6.8 and the formulation EL5 released upto 62.11% and ES5 releases complete drug 85.10%. EL5 and ES5 formulation further continued dissolution in pH 7.4 and it releases 79.1 & 93.01. Hence ES5 formulation showed better release. According to the zero-order equation the regression values of the formulations were 0.990 and 0.996 for EL5, ES5 respectively and hence it follows zero order. Higuchi plot shows R^2 values of 0.905 and 0.982 for EL5 and ES5. It shows that the mechanism of diffusion is not involved in the drug release. From the Korsmeyer Peppas equation, slope (N) values were 0.614, 0.716 for EL5 and ES5. It indicates that the formulation EL5 and ES5 shows Non-Ficikian diffusion.

Evaluation Parameters of Microsponges Loaded Ketoconazole Gel

The visual inspection of the gel (GES5) was done which stated that the gel was homogenous in nature with translucent and white in colour. The pH of the prepared ketoconazole gel (GES5) was 7.1 ± 0.34 as mentioned in Table 8. The amount of drug entrapped in the gel i.e. the drug content was found to be 3.33±1.615. The spreadability studies showed the spreadability values as 4.57±0.04.

Diffusion Studies and Kinetics of Drug Release from Ketoconazole Microsponges Gel (GES5)

The *In vitro* diffusion studies of the optimized ketoconazole gel (GES5) were performed for 510 mins which showed the drug release upto 96.21% as mentioned in Table 7. The regression values of the zero order and first order of the gel were 0.932 and 0.992 respectively which showed that the formulated ketoconazole gel (GES5) follows first order kinetics. The Higuchi plot shows R^2 value of 0.972, it follows Higuchi diffusion. From the Korsmeyer Peppas equation, slope N values were 0.814 hence it follows non-Ficikian diffusion as shown in Table 8.

FTIR Studies

In order to determine the various chemical interaction occurring between drug and excipients, FTIR studies were performed. Infrared spectrum of the pure drug and physical mixtures were obtained and analysed for principal peaks at 1511 (CNO_2), 3047 (CH), 1647 (C=O), 1073 (C-N), 1551 (C-NO_2). C-N stretching (amine and purine) as shown in Figure 14, Figure 15, Figure 16 and Figure 17.

From the above interpretation it was understood that there is no major shifting in the frequencies of above said functional groups of ketoconazole. This shows that there was no chemical interactions between ketoconazole and eudragit polymers which were utilized in the formulations.

SEM Analysis

The surface characterization and morphology of microsponges formulation were evaluated by the help of SEM analysis. It stated that the microsponges were uniform and spherical with no drug crystal surface. They were found to be highly porous when observed in the SEM images. The Microsponges which were prepared with PVA as surfactant had a very smooth surface with small pores as shown in Figure 18.

CONCLUSION

Ketoconazole microsponges were prepared with the help of two different polymers Eudragit L-100,

Eudragit S-100 in four various ratios (1:2, 1:3, 1:4,1:5). Evaluation tests like production yield, drug content and entrapment efficiency were performed and the formulation ES L containing Eudragit L-100 (1:5) and ES 5 containing Eudragit S-100 (1:5)showed better results. Formulations EL5 and ES5 were optimised and in vitro dissolution studies were performed. ES5 formulation shows maximum release up to 8 hours and can be used in the preparation of topical gel. Various drug release kinetic studies like Zero order and first order, higuchi and peppas plots were fitted and results showed that the optimised formulations of microsponges followed Zero order kinetics and showed Non fickian diffusion. The optimized gel formulation (GES5) was found translucent, white, homogenous and pH, drug content, Spreadability studies were done. The release studies data was plotted in the four kinetic models such as zero order, first order, higuchi and peppas plots. From the diffusion data of the drug, it was observed that formulations GES5 followed the first order kinetics with diffusion release mechanism. From the Korsmeyer Peppas equation, slope N value it followed Non-Ficikian diffusion. From the present investigation, it is could concluded that microsponges can be used as a useful and efficient formulation to improve drug delivery hence improving the bioavailability of the drug and also acts as an efficient carrier through the skin.

Conflict of Interest

The authors approves that there is no conflict of interest.

Funding Support

The authors hence declares that there is no financial support provided to conduct this study.

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