Current Research in Pharmaceutical Sciences
Available online at www.crpsonline.com

ISSN: 2250 – 2688

Received: 25/04/2016
Revised: 11/06/2016
Accepted: 22/06/2016

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Preparation and Evaluation of Ethosomal Gel of Clotrimazole for Fungal Infection by Mechanical Dispersion Method

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ABSTRACT

The present study investigates the use of ethosomal formulation of clotrimazole by mechanical dispersion method. In this work initially ethosomes of clotrimazole were prepared by using different concentration of soya lecithin and then optimized formulation batch was selected by determining Size and surface morphology , drug entrapment efficiency (DEE),*in-vitro* release study and the best formulation was used to prepare gel by using carbopol 934 as a gelling agent . pH and Viscosity were determined of Ethosom entrapped gel. The formulated ethosomal gel obtained was exploited to achieve a zero order release profile of Clotrimazole. DEE was obtained between 52.6 to 60% in best formulations This study disclosed that RE5* was the best promising formulation among all prepared formulations.

Keywords: Ethosomes, Gel, Clotrimazole, Efficiency.

1. INTRODUCTION

Ethosomes were developed by adding ethanol, phospholipids, and water. Ethanol is an efficient permeation enhancer that is believed to act by affecting the intercellular region of the stratum corneum. Ethosomes are soft malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration), and water. These formulations are soft vesicles represent vesicles carriers to enhance delivery through the skin[1-2].

Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and / or the systemic circulation. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization. Therefore, when integrated into a vesicles membrane; it gives the vesicle the ability to penetrate the stratum corneum. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than the conventional vesicles, although it has equivalent stability, allowing a more malleable structure and improves the drug distribution ability in the stratum corneum lipids[3].

Ethosomes are soft and flexible vesicle system mainly composed of phospholipids, water, poly glycol (propylene glycol 400) and a high concentration of alcohol (ethanol, isopropyl alcohol), usually about 20%, to 40% due to flexible lipid bilayer structure. The high concentration of ethanol brings increase in fluidity of lipid hence increase in permeability of skin and improves the drug penetration.

Clotrimazole a lipophilic and imidazole derivatives. Clotrimazole is a broad spectrum anti-fungal agent. It is used in the topical treatment of tinea infection like ringworm; 60-100% cure rates are reported with 2-4 weeks application an, a twice daily schedule. It is also effective in skin infection caused by *carynebacteria*. Topical and oral clotrimazole can be used in both adult and pediatric population. It may be used to treat the sickling of cell (related to sickle cell anemia). Ethosomes are soft and flexible vesicle system mainly composed of phospholipids, water, polyglycol (propyleneglycol) and a high concentration of alcohol (ethanol, isopropyl alcohol), usually about 20% to 40%. Owing to a flexible lipid bilayer, ethosomes are deformable and permeate deep into the skin, thus enhancing drug delivery.Moreover, they increase drug deposition in the skin and enhance its permeability in scar tissue[4-5].

Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic. That is why Ethosomes was chosen as a suitable carrier for the formulation of Clotrimazole entrapped gel. Ethosome formulations provide sustained delivery of drugs where ethosomes act as reservoir system for continues delivery of drugs [6].

2. MATERIALS AND METHODS

2.1 Drug and chemicals used

Clotrimazole was obtained as a gift sample from, Soya Lecithin ,Carbopol were purchased from Chemical Drug House Delhi. All other chemical were of Analytical Grade.

2.2 Preparation of clotrimazole Ethosomes

Ethosomes were formulated by mechanical dispersion method in which Soya phosphatidylcholine was dissolved in a mixture of chloroform: ethanol (3:1) v/v in round bottom flask. The organic solvent were removed by using rotary vacuum evaporator above lipid transition temperature (55 ±0.2°C) to form thin lipid film on the wall of the flask, followed by hydration (6h) with different concentration (20%, 30%, 40%, 50% w/v of ethanol in water) of hydro-ethanolic mixture. In this hydro-ethanolic mixture the concentration of ethanol and water was changed to observe changes in the batch no E5. It was then sonicated using bath sonicator to reduce the size of ethosomes.

2.3 Evaluation and characterization of ethosomes

2.3.1 Particle Size Analysis

The particle size and particle size distribution of ethosome was evaluated using optical microscope at 40x magnification. The prepared slide of ethosome and it was examined by an optical microscope and size of the ethosomes was measured using the pre-calibrated occularmicrometer at 40x magnification. About 25 ethosomes of each formulation were observed and average particle size was determined.

2.3.2 Shape and Surface Morphology

Shape and surface morphology of prepared ethosomes were observed in leica EC3. The prepared slide was observed at 10x magnification.

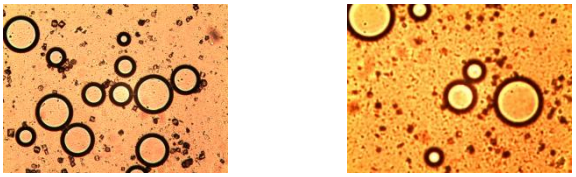

Figure1. Surface morphology of Prepared Ethosomes

2.3.3 Entrapment efficiency

The entrapment efficiency of ethosomes was measured by the ultracentrifuge method. Entrapment efficiency of ethosomal formulations was determined by separating the unentrapped drug from Vesicular preparations (2ml) containing fluconazole .Un-entrapped drug was separated by ultracentrifugation method. This vesicular suspension was diluted upto 10 ml with distilled water. Centrifugation was carried out at 1200 rpm for 20min.The supernatant liquid was analyzed for an un-entrapped drug by UV spectrophotometer at 238nm.

% Entrapment= Amount of drug in Entrapped/amount of Drug added ×100

2.3.4 Preparation of carbopol gel of clotrimazole ethosomes

The prepared and optimized 1% clotrimazole ethosomes batch E4 and E5 having good entrapment efficiency were incorporated into the gel base (Carbopol 934). 0.375g of carbopol 934 was weighed and soaked in given quantity of distilled water for 24 hr. After 24 hour carbopol was dispersed in 50ml of distilled water by using magnetic stirrer at 500 rpm for 2 hr. It was then neutralized with given quantity of Triethanolamine (4 ml) and stirred until a homogeneous gel was formed in Batch RE 4 and RE5.

2.3.5 pH of ethosomal gel

The pH measurements of the gel were carried out by using a digital pH meter by dipping the glass electrode completely into the semisolid formulation as to cover the electrode.

2.3.6 Viscosity of the ethosomal gel

The viscosity of gel was determined by using a Brookfield viscometer DVII model with T-Bar spindle in combination with a Heli path stand. Spindle T-bar was used for the measurement of viscosity of the gel. The viscosity was measured using 50 g of gel filled in a 100ml beaker. The T-bar spindle was lowered at the Centre taking care that spindle does not touch bottom of the beaker. The T-bar spindle was used for determining the viscosity of the gels. Which affect the viscosity was maintained during the process. The Heli path T- bar spindle was moved up and down giving viscosities at number of points along the path. The average of three readings taken in one minute was noted as the viscosity of the gel.

2.3.7 In vitro drug release study

In vitro permeation studies were carried out using franz diffusion cell. The release of drug determined by using the treated egg membrane mounted on upward into the donor compartment. 1 g of ethosomal gel formulation was applied on donor compartment and covered with petroleum jelly. Reservoir compartment was filled with 250 ml of pH 7.4 phosphate buffer. The study was carried out at 37± 0.5^{o}C and at a speed of 50 rpm for 12 hour. The solutions of the receptor side were stirred by externally driven magnetic bars using magnetic stirrer at 50 rpm. 5 ml sample was withdrawn at every 1 hr interval and replaced by fresh saline PBS 7.4 pH to maintain the simulated condition. The drug concentration was recorded at 238 nm using UV spectrophotometer.

3. RESULTS AND DISCUSSION

3.1 Particle size

The size range of ethosomes were in the range of 3-15µm. The particle size was found to be increased with increase in soya lecithin concentration but as the ethanol concentration was increased the size range of ethosome was decreased, ethanol probably caused an alteration of the net charge of the system and conferred it some extent of steric stabilization that might finally lead to a reduction in the vesicle size. Depending upon the size range the order for the formulation was RE 1>RE 3>RE 2>RE 5>RE 4>RE 6. As shown in table no 2.

3.2 Shape and surface morphology

The shape and morphology was studied using Leica EC 3. The prepared slide was observed at 10x magnification . Smooth surface of prepared ethosomes was observed in this study. It was also found that there were no that when the soya lecithin concentration was increased the size of ethosome also increased. The size of ethosome was also significant difference in the surface morphology of prepared Ethosomes .As shown in fig 1.

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3.3 Entrapment efficiency

The values were obtained for entrapment efficiency from the formulations revealed that by increasing phospholipid concentration it was observed that the entrapment efficiency decreases whereas on keeping phospholipid concentration constant and increasing ethanol results in increasing entrapment efficiency. Entrapment efficiency (average) of the formulations containing 1%w/v of soya lecithin and ethanol 30% v/v (i.e, RE 2) was lower than that of the formulations having 1.5% w/v soya lecithin and ethanol 30% v/v (i.e, RE 3). Whereas batch RE 5 having 2% w/v soya lecithin and 30%v/v shows better entrapment efficiency due to presence of high ethanol concentration as shown in table no 2.

3.4 *In vitro* drug release study

In vitro drug release study was carried out for RE 4 and RE 5 formulation as their entrapment efficiency was better than RE 1, RE 2, RE 3,RE and RE 6.RE 4 showed maximum releases and so its drug release was studied using different kinetic models. From the drug release study of RE 5, release kinetics was studied using Zero order, First order, Higuchi release kinetic and Korsemeyer peppas kinetic model. The regression value of each of these release kinetics were calculated and compared. As shown in table no 4. The data revealed that the release pattern of formulation best fitted for Korsemeyerpeppas kinetic models. *In vitro* drug release study showed that the maximum drug release of 63.34% for RE 5 in 8 hours as shown in Table no 5.

3.5 pH of the ethosomal gel

Final optimized batches RE 4 and RE 5 were incorporated into the gel and the pH was calculated. It was observed that batch RE 4 having 35% w/w ethanol shows pH at 6.4 whereas RE 5 having 30% w/v ethanol shows pH 6.6 due to presence of 2% w/w soya lecithin as shown in table no 3.

3.6 Viscosity of the ethosomal gel

The viscosity of the batch RE 4 and RE 5 were calculated. The viscosity was maintained so that it could spread easily. The viscosity of batch RE 4 was 3200 cps and RE 5 was 4100 cps at the shear rate of 100 rpm as shown in table no 4.

Table No.1: Different formulation of Ethosomes prepared by mechanical dispersion method

Batch no.	Drug	Composition(%w/w)				Average Size	Result
		Soya lecithin %	Cholesterol %	Ethanol %	Water (%)		
E1	1%	0.5	0.1	20	Upto1	6.27±0.1	Ethosome were formed but not stable
E2	1%	1	0.1	30	Upto100ml	6.21±0.2	Ethosomes were Formed but surface was irregular
E3	1%	1.5	0.1	30	Upto100ml	8.23±0.1	Ethosomes were formed and the surface was better than batch 2
E4	1%	1.5	0.1	35	Upto100l	10.0±0.2	Ethosomes were formed with good surface morphology
E5	1%	2	0.1	30	Upto100ml	15.0±1.2	Ethosomes were formed and the vesicle size was more than batch no.4
E6	1%	2	0.1	40	Upto100ml	12.0±1.4	Ethosomes were formed but not good

Table No. 2: Average Vesicles size and Entrapment efficiency of Ethosomes

S.No	Formulation code	Average Vesicles(µm) Mean size±SD	Drug entrapped efficiency%
1	E1	6±0.1	33.26%
2	E2	6±0.2	46.98%
3	E3	8±0.12	49.24%
4	E4	10±0.21	52.62%
5	E5	15±1.2	60.06%
6	E6	12±1.4	49.37%

Table No. 3: pH of batch RE 4 and RE5

S.no	Batch name	pH
1	RE 4	6.4
2	RE 5	6.6

Table No. 4: Viscosity of batch RE 4 and RE 5

S.no	Shear rate RPM	Batch name	Viscosity(cps)
1	100	RE 4	3200
2	100	RE 5	4100

Table No. 5: in vitro cumulative % drug release of RE5

Time (hour)	% cumulative drug release
0	0
1	14.16
2	19.84
3	36.02
4	37.90
5	42.54
6	46.09
7	59.76
8	63.34

Table No. 6: Regression co-efficient (r^2) values of different kinetic models for formulation RE 5

Release kinetics Model	Regression value (r^2)
Zero order	0.9629
First order	0.1383
Higuchi	0.9522
Korsemeyer- Peppas	0.9681

4. CONCLUSION

An effort was made to formulate drug entrapped Ethosomes Clotrimazole ,It was observed during formulation of ethosomes that formulation RE 4 and RE 5 were passed all the evaluation parameters and finally incorporated into gel and *in vitro* drug release was perrformed. The release rate was dependent on ethanol andphosholipid concentration. RE 5 shows better release and followed korsemeyer-peppas kinetic model with 63.34% Cumulative drug release. The pH and viscosity of the final formulation was observed to be 6.4 and 6.6, 3200 cps and 4100 cps respectively for batch RE 4 and RE 5. The pH was affected due to the soya lecithin concentration. These results are very satisfactory and hence confirmed that ethosomes are a very efficient carrier for topical administration of clotrimazole.

5. ACKNOWLEDGEMENT

Author is thankful to Dr A.K. Pathak, Head, Department of Pharmacy to provide research facility and Dr A.K. Mishra for constant support and guidance.

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