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Research Article

FORMULATION DEVELOPMENT AND CHARACTERIZATION OF CLOTRIMAZOLE TRANSFERSOMAL GEL FOR EFFECTIVE TREATMENT OF FUNGAL DISEASE

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ABSTRACT

Transdermal delivery system generally shows better regulation of blood levels, decreased occurrence of systemic toxicity, no first pass hepatic metabolism, and higher compliance as compared with conventional formulations. Transdermal treatment systems are characterized as self containing and discrete dosage types, which supply the medication with a regulated rate of systemic circulation through the skin if applied to the intact skin. The aim of present work to develop transdermal drug delivery system, transfersomes incorporated gel of Clotrimazole for effective treatment of Candidiasis. Prepared formulations of Transfersomes were optimized on basis of vesicle size, shape, surface charge and entrapment efficiency. Average vesicle and zeta potential of optimized formulation F-12 was found 145.58nm and -38.25 mV respectively. Prepared gel was prepared and evaluated for viscosity, % assay, extrudability, spreadability and drug release study. It was found that viscosity of prepared gel was 3150±12 cps, % assay was 99.45±0.12, Extrudability was 175g and Spreadibility (g.cm/sec) was found that 12.32±0.42 (g.cm/sec) respectively. *In vitro* drug release from Transfersomes was carried out using Franz diffusion cell method and found 98.12% in 10 hr. In first 30 min., it was 182±4 drug release which is slightly high. It was due to the release of free drug present in bag after leaching from transfersomes. Drug release from transferosomal formulation was found in very sustained and controlled manner.

Keywords: Transdermal delivery, Clotrimazole, Transfersomes, Evaluation, Candidiasis.

1. INTRODUCTION

The prevalence of superficial fungal infections of the skin, hair, and nails has risen globally. In undeveloped and underdeveloped countries, it is believed that roughly 40 million people have been infected with fungi. Due to compromised immune function, the course of fungal infections can be fast and serious [1]. One of the most common causes of tinea and onchomycosis is dermatophytes. Candida infections are also among the most common fungal infections of the skin. When the immune system is weakened, candida can further infect deeper tissues and blood, resulting in life-threatening systemic candidiasis.

Topical treatment of fungal infections has various advantages, including the ability to target the infection site, reduced risk of systemic adverse effects, improved treatment efficacy, and high patient compliance. A range of topical antifungal agents have been employed in the treatment of various dermatological skin infections. These antifungal medicines are currently accessible in creams, gels, lotions, and sprays in traditional dose forms [2].

The effectiveness of topical antifungal treatment is determined on drug penetration through the target tissue. As a result, the effective medication concentration levels in the skin should be obtained. When antifungals are applied topically, the drug components must penetrate through the stratum corneum; the skin's outermost layer, to reach the lower layers, notably the viable epidermis [3]. New carrier systems for licensed and investigational medications are being developed as alternate techniques for topical treatment of fungal infections of the skin. Antifungal chemicals can be delivered to the skin more effectively using carriers such as colloidal systems and vesicular carriers. Gregor Cevc coined the term Transfersome and developed the fundamental model in 1991. Since that time, a great deal of research has been done on these adaptable vesicles under several names such as adaptable vesicles, ethosomes, and so on. A Transfersome is a tremendously versatile, very responsive, and multidimensional whole in the fullest sense. Its preferred shape is a highly flexible vesicle with a watery center surrounded by a perplexing lipid bilayer. Transfersome is a trademarked name used

by the German company IDEA AG to refer to its restricted medicine delivery technology. The name 'transferre' comes from the Latin word 'transferre,' which means 'to convey over,' and the Greek word 'soma,' which means 'body'. A Transfersome transporter is a synthetic vesicle that looks like a typical cell vesicle. It is appropriate for targeted and regulated pharmaceutical delivery in this manner [4-6]. Transfersomes are vesicles with an ultra-adaptable layer that are self-improved totals. These vesicular transfersomes are more flexible than normal liposomes, making them suitable for skin penetration [7-8].

A transfersome is a complex aggregate that is extremely flexible and stress-responsive. Its preferred form is an ultradeformable vesicle with an aqueous core and a complex lipid bilayer surrounding it. Water-filled colloidal particles are known as vesicles. The walls of these capsules are made up of bilayers of amphiphilic molecules (lipids and surfactants). In the case of topical formulations, these vesicles serve as a depot for the sustained release of active substances, as well as a rate-limiting membrane barrier for the control of systemic absorption in the case of transdermal formulations [9-12]. Clotrimazole, a broad spectrum less toxic imidazole antifungal agent is widely used to treat Candidiasis. It acts by inhibiting cytochrome 14α-demethylase enzyme of the fungal cells responsible for cell wall synthesis. Chemically, clotrimazole is 1-((2-chlorophenyl) diphenylmethyl)-1H-imidazole, insoluble in water (0.49 mg/L) with Log P of 6.1 and pKa 6.7. It is the first oral azole approved for fungal infections; however, it is not used as an oral agent due to its limited oral absorption and systemic toxicity. Transdermal delivery system generally shows better regulation of blood levels, decreased occurrence of systemic toxicity, no first pass hepatic metabolism, and higher compliance as compared with conventional formulations. Transdermal treatment systems are characterized as self containing and discrete dosage types, which supply the medication with a regulated rate of systemic circulation through the skin if applied to the intact skin. The aim of present work to develop transdermal drug delivery system, transfersomes incorporated gel of Clotrimazole for effective treatment of Candidiasis.

2. MATERIAL AND METHODS

2.1. Material

Clotrimazole and Soya PC was purchased from Himedia Laboratory, Mumbai. Ethanol, chloroform and carbopol-934 purchased from CDH chemical Pvt. Ltd. New Delhi. Dialysis membrane of Mol Wt cutoff 1200 was purchased from Himedia Laboratory, Mumbai. Demineralized and double distilled water was prepared freshly and used whenever required. All other reagents and chemicals used were of analytical grade.

2.2. Formulation, development clotrimazole loaded transfersomes

2.2.1. Preparation of clotrimazole loaded transfersomes

Required quantities of Soya PC and surfactant were taken in a round bottom flask and dissolved in ethanol by shaking. The thin film was formed by rotary evaporation by using rotary evaporator for 15 minutes at 25°C, 600mm/hg pressure and 100rpm. The solvent was then evaporated under a nitrogen gas stream [13-14]. The lipid film was placed in a desiccator for at least 12 h to remove any remaining solvent. Clotrimazole was dissolved in 10ml, 7.4 pH (simulated tear fluid) which was heated to 55°C. The film was then hydrated with the heated buffer by hand shaking for half an hour. Then the mixture was stirred for half an hour in orbital shaker. THE transferosomes were then observed under microscope. Transferosomal suspension was stored in refrigerator at 40°C.

2.2.2. Preparation of Gel Base

Carbopol 934 (1%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.8. Transferosomal preparation corresponding to 0.1% w/w of clotrimazole was incorporated into the gel base to get the desired concentration of drug in gel base.

2.3. Optimization of transfersomes

2.3.1. Optimization of ratio of lipid and surfactant

In the transferosomal formulation, the ratio of lipid and surfactant was optimized by taking their different ratio such as 9:1, 8:2, 7:3 and 6:4 % ratio and all other parameters were kept remain constant. The prepared formulations were optimized on the basis of average vesicle size and % entrapment efficiency [15].

2.3.2. Optimization of drug concentration:

Drug concentration was optimized by taking different

concentration of drug and prepared their formulation and all other parameter such as Soya PC, stirrer time kept remains constant. The formulation optimized on the basis of entrapment efficiency and average vesicle size [16].

2.3.3. Optimization of stirrer duration

Stirring duration was optimized by duration the formulation for different time duration *i.e* 5, 10 and 15 min. The optimization was done on the basis of average vesicle size, and % Entrapment efficiency [17].

Table 1: Optimization of ratio of lipid concentration

Formulation code	Soya PC: Span 20 (% w/v)	Ethanol	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
F1	9:1	10	1.0	330.32	65.25
F2	**8:2**	**10**	**1.0**	**256.56**	**73.32**
F3	7:3	10	1.0	298.85	63.31
F4	6:4	10	1.0	310.25	60.45

Table 2: Optimization of ethanol concentration

Formulation code	Soya PC (% w/v)	Ethanol	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
F5	8:2	5	1.0	295.56	65.58
F6	**8:2**	**10**	**1.0**	**220.14**	**75.65**
F7	8:2	15	1.0	265.58	63.32
F8	8:2	20	1.0	295.65	64.85

Table 3: Optimization of drug concentration

Formulation code	Soya PC (% w/v)	Drug (% w/v)	Ethanol (ml)	Average vesicle size (nm)	% Entrapment efficiency
F9	8:2	1.0	10	165.58	76.65
F10	8:2	1.5	10	195.65	69.52
F11	8:2	2.0	10	173.32	70.32

Table 4: Optimization of Stirrer duration

Formulation code	Soya PC: (% w/v)	Drug (% w/v)	Stirrer duration (min)	Average vesicle size (nm)	% Entrapment efficiency
F12	**1.0**	**1.0**	**5**	**145.58**	**78.85**
F13	1.0	1.0	10	135.65	65.65
F14	1.0	1.0	15	126.65	58.85

2.4. Characterization of Clotrimazole loaded transfersomes

2.4.1. Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the transfersomes was based on the Zeta potential that was calculated according to Helmholtz-Smoluchowsky from their electrophoretic mobility. For measurement of Zeta potential, a Zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9 % NaCl adjusted to a conductivity of 50 lS/cm [18].

2.4.2. Entrapment efficiency

One milliliter of transfersomes suspension was centrifuged at 15.000 rpm for 1 h to allow to separate the entrapped drug from the un-entrapped drug. After removal of the supernatant, the sediment was lysed using methanol and then analyzed spectrophotometrically at 260nm using a UV spectrophotometer (Labindia 3000+). The Entrapment efficiency % of drug in the prepared transfersomes was calculated applying the following equation:

% Entrapment Efficiency= {(Theoretical drug content-Practical drug content)/ Theoretical drug content) x100

2.4.3. In vitro drug diffusion study

In vitro drug release of formulated tranfersomes was performed by applying the dialysis diffusion technique, using the dissolution test apparatus. Phosphate buffer (pH 7.4) was used as the dissolution medium. The dialysis method was applied using cellulose acetate dialysis membrane of 12,000-14,000 molecular weight cut off. This membrane assures the permeation of the drug with retention of tranfersomal vesicles. The membrane was soaked in artificial tears for 12 h before use. Four ml of tranfersomal dispersion was placed in a glass cylinder having a length of 8 cm and diameter of 1 cm and dialysis membrane was fixed to opening of glass cylinder by a thread. Each glass cylinder was attached to the shaft of the dissolution apparatus (USP Dissolution tester, Labindia DS 8000) and descended down into a 100 ml beaker containing 50 ml of as dissolution medium without touching the bottom surface of the beaker. The beaker was then placed into vessels of dissolution apparatus that contained about 100 ml of water to keep temperature at 34±0.5°C. The glass cylinders were adjusted to rotate at a constant speed of 20 rpm. One ml of dissolution medium was withdrawn at predetermined time intervals (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6 h).

The samples were replaced with fresh dissolution medium to maintain constant volume. Drug concentrations in samples were analyzed using spectrophotometrically at wavelength of drug 260nm. The release experiments were carried out in triplicates and the mean±SD were recorded. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling [19-20].

2.5. Characterization of Transfersomes containing Gel

2.5.1. Measurement of Viscosity

Viscosity measurements of prepared topical Transfersomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm; viscosity.

2.5.2. pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted.

2.5.3. Drug Content

Accurately weighed equivalent to 100 mg of topical transfersomal gel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λ_{max} 260nm.

2.5.4. Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load. More the quantity of gel extruded shows better extrudability. It was determined by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

2.5.5. Spreadibility

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadibility, placed 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 6cm upon adding 20g of weight was noted. Good spreadibility show lesser time to spread.

Spreadability (g.cm/sec) = (Weight tide to upper slide x Length moved on the glass slide)/Time taken to slide

2.5.6. In vitro drug diffusion study

The *In-vitro* diffusion study was carried by using Franz Diffusion Cell. Egg membrane was taken as semi permeable membrane for diffusion. The Franz diffusion cell has receptor compartment with an effective volume approximately 30mL and effective surface area of permeation 3.14sq.cms. The egg membrane was mounted between the donor and the receptor compartment. A two cm^2 size patch was taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium was phosphate buffer (pH 7.4). The receptor compartment was surrounded by water jacket so as to maintain the temperature at 32±0.5°C. Heat was provided using a

thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by Teflon coated magnetic bead which was placed in the diffusion cell. During each sampling interval, samples were withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn were analyzed spectrophotometrically at wavelength of drug 260nm.

2.6. Antifungal activity of optimized transfersomes gel

The well diffusion method was used to determine antifungal activity of clotrimazole loaded transfersomes using standard procedure [21]. There were 2 concentrations used which were 10 µg/ml and 20 µg/ml transfersomes in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 28°C for 48 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

3. RESULTS AND DISCUSSION

Prepared formulations of Transfersomes were optimized on the basis of vesicle size, shape, surface charge and entrapment efficiency. Vesicle size of Transfersomes were examined under trinocular microscopic (magnification 400X) and also determined by light scattering method (Malvern Zetasizer, ZEM 5002, and UK) and found that average vesicle size of optimized formulation F-12 was 145.58nm. Zeta potential was -38.25 (It was observed that the vesicles size of transfersomes was increased with increasing the concentration of phosphotidylcoline and similarly vesicle size). There was no significant difference in average vesicle size observed with increasing the drug concentration. But by increasing the stirrer time, the vesicle size was decreased from 145.58 to 126.65 after 15 min of stirring.

% Entrapment efficiency of optimized transferosomal formulation (F-12) was found to be 78.85%. It was observed that the percent drug entrapment was decreased with increasing the concentration of ethanol and on increasing the time of stirring. It is due to leaching out of the drug from vesicles on increasing the mechanical force by stirring and size reduction. It was clearly shown when formulation was stirred for 5, 10, 15 min, the % Entrapment efficiency was 78.85, 65.65 and 58.85 respectively. The 5 min was selected as optimized time for stirrer because it provided the required size of vesicle 145.58 nm and good % Entrapment efficiency *i.e* 78.85. The F-12 formulation was selected as optimized formulation.

Prepared gel was prepared and evaluated for viscosity, % assay, extrudability, spreadability and drug release study. It was found that viscosity of prepared gel was 3150±12 cps, % assay was 99.45±0.12, Extrudability was 175g and Spreadibility (g.cm/sec) was found as 12.32±0.42 (g.cm/sec) respectively. *In vitro* drug release from transfersomes was carried out using Franz diffusion cell method and found 98.12% in 10 hr. In first 30 min., it was 182±4 drug release which is slightly high. It was due to the release of free drug present in bag after leaching from transfersomes. Drug release from transferosomal formulation was found in very sustained and controlled manner.

Table 5: *In vitro* drug release study of prepared transfersomal formulation F12, F13 and F14

S. No.	Time (hr)	% Cumulative Drug Release		
		F12	F13	F14
1	0	0.0	0.0	0.0
2	0.5	16.65	11.56	12.23
3	1	22.23	20.23	16.65
4	1.5	29.98	25.65	23.32
5	2	36.65	32.23	30.14
6	2.5	46.65	43.32	36.65
7	3	57.74	51.14	45.58
8	3.5	63.32	52.32	49.95
9	4	68.89	65.56	53.32
10	4.5	72.32	67.78	55.65
11	5	79.98	68.85	58.85
12	6	81.12	69.95	58.98

Table 6: *In-vitro* drug release data for F12

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	16.65	1.221	83.35	1.921
1	1	0	22.23	1.347	77.77	1.891
1.5	1.225	0.176	29.98	1.477	70.02	1.845
2	1.414	0.301	36.65	1.564	63.35	1.802
2.5	1.581	0.398	46.65	1.669	53.35	1.727
3	1.732	0.477	57.74	1.761	42.26	1.626
3.5	1.871	0.544	63.32	1.802	36.68	1.564
4	2	0.602	68.89	1.838	31.11	1.493
4.5	2.121	0.653	72.32	1.859	27.68	1.442
5	2.236	0.699	79.98	1.903	20.02	1.301
6	2.449	0.778	81.12	1.909	18.88	1.276

**Average of three reading (mean ±SD)*

Table 7: *In-vitro* drug release data for F13

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	11.56	1.063	88.44	1.947
1	1	0	20.23	1.306	79.77	1.902
1.5	1.225	0.176	25.65	1.409	74.35	1.871
2	1.414	0.301	32.23	1.508	67.77	1.831
2.5	1.581	0.398	43.32	1.637	56.68	1.753
3	1.732	0.477	51.14	1.709	48.86	1.689
3.5	1.871	0.544	52.32	1.719	47.68	1.678
4	2	0.602	65.56	1.817	34.44	1.537
4.5	2.121	0.653	67.78	1.831	32.22	1.508
5	2.236	0.699	68.85	1.838	31.15	1.493
6	2.449	0.778	69.95	1.845	30.05	1.478

**Average of three reading (mean ±SD)*

Table 8: *In-vitro* drug release data for F14

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	12.23	1.087	87.77	1.943
1	1	0	16.65	1.221	83.35	1.921
1.5	1.225	0.176	23.32	1.368	76.68	1.885
2	1.414	0.301	30.14	1.479	69.86	1.844
2.5	1.581	0.398	36.65	1.564	63.35	1.802
3	1.732	0.477	45.58	1.659	54.42	1.736
3.5	1.871	0.544	49.95	1.699	50.05	1.699
4	2	0.602	53.32	1.727	46.68	1.669
4.5	2.121	0.653	55.65	1.745	44.35	1.647
5	2.236	0.699	58.85	1.770	41.15	1.614
6	2.449	0.778	58.98	1.771	41.02	1.613

**Average of three reading (mean ±SD)*

Table 9: Characterization of Optimized formulation of transfersomes

Characterization	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F-12	145.58	78.85	-38.25

Table 10: Characterization of gel based formulation of prepared gel containing clotrimazole loaded Transfersomes

Formulation	Viscosity* (cps)	Assay* (%)	Extrudability* (g)	Spreadability* (g.cm/sec)
Gel formulation	3150±12	99.45±0.12	182±4	12.32±0.42

**Average of three determination*

Table 11: *In vitro* drug release study of prepared gel formulation

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	15.65
2	1	26.65
3	1.5	38.85
4	2	46.65
5	4	59.98
6	6	72.23
7	8	88.98
8	10	98.12

**Average of three determination*

Table 12: Regression analysis data of transfersomal gel formulation

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
			R^2	
Optimized gel formulation	0.954	0.897	0.990	0.977

4. CONCLUSION

Transfersomes were prepared and optimized on the base of average vesicle size and % drug entrapment. The optimized formulation was further incorporated with gel base (Carbopol gel) and characterized for their viscosity, pH, % drug content, extrudability, spreadability and drug release study. It can be concluded that prepared gel containing clotrimazole loaded transfersomal formulation was optimized and can be of used for topical preparation for its antifungal effect.

Conflict of interest

None declared

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