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Research Article

FORMULATION DEVELOPMENT AND EVALUATION OF TRANSFERSOMES OF ECONAZOLE FOR EFFECTIVE FUNGAL TREATMENT

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ABSTRACT

The aim of the present work is to formulate and characterize Econazole loaded transfersomal gel for antifungal activity. Econazole is a broad-spectrum imidazole antifungal agent that belongs to BCS Class II. Due to poor solubility, Econazole is incompletely absorbed after oral administration and bioavailability vary among individuals. Topical treatment of fungal infections is usually preferred, but the barrier is to cross stratum corneum, so formulating the drug in Transfersomes solved this problem. Prepared formulations of transfersomes were optimized on basis of vesicle size, shape, surface charge and entrapment efficiency. In order to predict and correlate the *in vitro* econazole permeation behavior from these transfersomes through semi-permeable membrane, it is necessary to fit into a suitable mathematical model. The *in vitro* drug permeation data from formulation F-12 containing econazole through semi-permeable membrane was evaluated kinetically by various mathematical models like zero-order, first-order, Higuchi, and Korsmeyer-Peppas Model. The results were obtained which showed that transfersomal gel was a promising candidate for transdermal delivery with targeted and prolonged release of a drug.

Keywords: Econazole, Transfersomes, Formulation Development, Evaluation.

1. INTRODUCTION

The incidence of superficial fungal infections of skin, hair and nails has been increased worldwide. It has been estimated that about 40 million people have suffered from fungal infections in developing and under developed nations. The progression of fungal infections can be rapid and serious due to compromising with immune function [1]. *Dermatophytes* are one of the most frequent causes of *tinea* and o*nchomycosis*. Candida infections are also among the most widespread superficial cutaneous fungal infections even, *candida* can invade deeper tissues as well as the blood which leads to life-threating systemic candidiasis, when the immune system is weakened. Topical treatment of fungal infections has several superiorities including, targeting the site of infection, reduction of the risk of systemic side effects, enhancement of the efficacy of treatment and, high patient compliance. Different types of topical effective antifungal compounds have been used in the treatment of a variety of dermatological skin infections. Currently, these antifungal drugs are commercially available in conventional dosage forms such as creams, gels, lotions and sprays [2].

The efficiency of the topical antifungal treatment depends on the penetration of drugs through the target tissue. Hence, the effective drug concentration levels should be achieved in the skin. In topical administration of antifungals, the drug substances should pass the *stratum corneum*, which is the outermost layer of the skin, to reach lower layers of the skin, particularly into *viable epidermis* [3]. Development of alternative approaches for topical treatment of fungal infections of skin encompasses new carrier systems for approved and investigational compounds. Delivery of antifungal compounds into skin can be enhanced with the carriers including colloidal systems, vesicular carriers, and nanoparticles. The term transfersome and the basic model were presented in 1991 by Gregor Cevc. From that point forward, tremendous measure of research is going on worldwide on these versatile vesicles under different titles like adaptable vesicles, ethosomes, and so forth. Its favored shape is an ultra deformable vesicle having a watery center encompassed by the mind boggling lipid bilayer. Transfersome is a term enlisted as a trademark by the German organization IDEA AG, and utilized by it to allude to its restrictive medication conveyance innovation. The name signifies "conveying body", and is duplicated from the Latin word 'transferre', signifying 'to

convey over', and the Greek word 'soma', for a 'body'. A transfersome transporter is a manufactured vesicle and takes after the characteristic cell vesicle. In this way it is appropriate for focused and controlled medication delivery [4-5]. Transfersomes are vesicles, which are self-improved totals with ultra-adaptable layer. These vesicular transfersomes are more flexible than the standard liposomes and in this manner appropriate for the skin penetration [7-8].

Occurrence of skin fungal infections is increasing nowadays and their presence is more prominent in patients suffering from immunocompromised diseases like AIDS. Skin fungal infections are a major cause of visits by patients to dermatology clinics. Although, a large number of antifungal agents are available for treatment of skin fungal infections, but, their toxic profile and physicochemical characteristics reduce therapeutic outcome.

When these antifungal agents are delivered topically using conventional formulations like creams and gels, they may cause various side effects like redness, burning, and swelling at the site of application. Econazole nitrate (1-[2-(4-chlorophenyl) methoxy]-2-(2, 4-dichlorophenyl) ethyl)-1H-imidazole mononitrate is a broad-spectrum imidazole antifungal drug mainly used to treat skin infections such as candidiasis. Econazole nitrate belongs to the BCS class (IV) so it has both rate-limited release and permeability. Econazole nitrate blocks the ergesterol synthesis thus it leads to prevention of fungal cell multiplication. Econazole nitrate is commercially available as 1% cream and 1% liposomal gel. Conventional liposomes have poor penetration through the skin, which can be improved by modifying their bilayer composition. Transfersomes show enhanced deformability due to the weakening of their lipid bilayers because of edge activators. Transfersomes have higher skin penetration compared to conventional liposomes due to their higher deformability and they can easily cross through the pores having diameter 5-10 times less compared to their own diameter. To avoid the problem associated with conventional dosage form and to reduce side effect associated with antifungal drug like econazole, transfersomes have been designed to overcome the problem of release permeability and side effects.

2. MATERIAL AND METHODS

2.1. Material

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

2.2. Preparation of Econazole 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 [9]. The lipid film was placed in a desiccator for at least 12 h to remove any remaining solvent. Econazole was dissolved in 10 ml, phosphate buffer pH 7.4 and heated up to 55°C. The film was then hydrated with the heated buffer by hand shaking for half an hour. The mixture was stirred for half an hour in orbital shaker and the transferosomes were observed under microscope. Transferosomal suspension was stored in refrigerator at 40°C. Total Fourteen formulations were prepared and optimization of transfersomes was done by optimization of lipid concentration, optimization of ethanol concentration, optimization of drug concentration and optimization of stirrer duration. The prepared formulation was evaluated for vesicle size, entrapment efficiency, zeta potential and *in vitro* drug release and anti-fungal activity.

2.3. Characterization of econazole loaded transfersomes

2.3.1. Surface charge and vesicle size

The vesicles size, 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 and 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 [10].

2.3.2. 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 [11]. After removal of the supernatant, the sediment was lysed using methanol and then analyzed spectrophoto-

metrically at 224nm using a UV spectrophotometer (Labindia 3000+). The Entrapment efficiency % of drug in the prepared transfersomes was calculated applying the following equation:

% Entrapment Efficiency = {(Therotical drug content-Practical drug content)/Therotical drug content} ×100

2.3.3. 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. Artificial tears fluid of 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 50 ml beaker containing 10 ml of artificial tears 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 224nm. 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 [12-13].

2.3.4. Antifungal activity of optimized transfersomes

The well diffusion method was used to determine antifungal activity of Econazole loaded transfersomes using standard procedure [14]. There were 2 concentrations used which are 10 µg/ml and 20 µg/ml transfersomes in antibiogram studies. The standard drug fluconazole and optimized formulation of prepared transfersomes (test) was taken into cups bored into sterile potato dextrose agar previously seeded with *Candida albicans*. The plate was incubated for 48 h at 25°C after allowing diffusion of formulation for 2 h. The zones of inhibition were measured in mm after 48 h for the test and standard.

2.4. Stability Studies

Stability study was carried out for drug loaded transfersomes at two different temperatures *i.e.* refrigeration temperature (4.0±0.2°C) and at room temperature (25-28±2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any physical changes and drug content.

3. RESULTS AND DISCUSSION

Prepared formulations of transfersomes were optimized on 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 145.25nm. Zeta potential was -42.12 (It was observed that the vesicles size of transfersomes was increased with increasing the concentration of phosphotidylcholine and similarly vesicle size. No significant difference in average vesicle size was observed with increasing the drug concentration. But in increasing the stirrer time, the vesicle size was decreased from 195.45to 195.45 after 15 min of stirring (table 1).

% Entrapment efficiency of optimized transferosomal formulation (F-12) was found to be 80.25%. It was observed that the percent drug entrapment decreased with increasing the concentration of ethanol and on increasing the time of stirring. It is due to the leaching out 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 80.25, 65.56 and 55.41. The 5 min is selected as optimized time for stirrer because it provided the required size of vesicle *i.e.* 145.25nm and good % entrapment efficiency *i.e.* 80.25. The F-12 formulation was selected as optimized formulation shown in table 3.

In order to predict and correlate the *in vitro* econazole permeation behavior from these transfersomes through semi-permeable membrane, it is necessary to fit into a suitable mathematical model. The *in vitro* drug

permeation data from formulation F-12 containing econazole through semi-permeable membrane was evaluated kinetically by various mathematical models like zero-order, first-order, Higuchi, and Korsmeyer-Peppas Model. The results of the curve fitting into these above mentioned mathematical models indicate the *in vitro* drug permeation behavior of transfersomes (F12). When respective correlation coefficients were compared, it followed the zero-order kinetics ($R^2 = 0.979$) over a period of 6 h. This indicates that the *in vitro* econazole permeation behavior from transfersomes was in controlled release manner throughout the *in vitro* econazole permeation study (table 4-6). Stability study data revealed that the optimized formulation (F-12) was stable after 3 month of storage at 4°C while at 25-28±2°C, the formulation was found unstable. Stability of formulation was observed on the basis of % drug remained, average vesicles size and physical appearance.

Table 1: Results of % average vesicle size

Formulation code	Average vesicle size (nm)	% Entrapment efficiency
Optimization of lipid concentration		
F1	325.65	65.56
F2	**220.32**	**73.32**
F3	315.56	69.98
F4	295.65	61.12
Optimization of ethanol concentration		
F5	315.25	66.54
F6	**195.45**	**75.56**
F7	285.45	70.21
F8	265.56	63.32
Optimization of drug concentration		
F9	**155.45**	**78.85**
F10	220.12	63.32
F11	265.45	66.45
Optimization of Stirrer duration		
F12	**145.25**	**80.25**
F13	195.45	65.56
F14	120.23	55.41

Table 2: Characterization of optimized formulation of transfersomes

Characterization	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F-12	145.25	80.25	-42.12

Table 3: *In-vitro* drug release study of prepared transfersomal formulation F12

S. No.	Time (hr)	% Cumulative Drug Release
1	0	0.0
2	0.5	20.25
3	1	25.65
4	1.5	28.89
5	2	34.45
6	2.5	40.25
7	3	45.65
8	3.5	53.12
9	4	61.14
10	4.5	72.23
11	5	83.32
12	5.5	89.98
13	6	96.65

Table 4: *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	20.25	1.902	79.75	1.902
1	1	0	25.65	1.871	74.35	1.871
1.5	1.225	0.176	28.89	1.852	71.11	1.852
2	1.414	0.301	34.45	1.817	65.55	1.817
2.5	1.581	0.398	40.25	1.776	59.75	1.776
3	1.732	0.477	45.65	1.735	54.35	1.735
3.5	1.871	0.544	53.12	1.671	46.88	1.671
4	2	0.602	61.14	1.590	38.86	1.590
4.5	2.121	0.653	72.23	1.444	27.77	1.444
5	2.236	0.699	83.32	1.222	16.68	1.222
5.5	2.345	0.74	89.98	1.001	10.02	1.001
6	2.449	0.778	96.65	0.525	3.35	0.525

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

Table 6: Regression analysis data of transfersomal formulation F12

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas
			R^2	
F12	0.979	0.796	0.918	0.545

Table 7: Anti-fungal activity of standard and optimized transfersomes

S. No.	Fungus	Drug/ Formulation	Zone of inhibition (mm)
1.	*Candida albicans*	Fluconazole (20 µg/ml)	15±0.47
2.		Transfersomes (10 µg/ml)	13±0.86
3.		Transfersomes (20 µg/ml)	12±0.94

4. CONCLUSION

In this present work, Econazole loaded transfersomes were successfully formulated by the thin-film hydration method. Results proved that particle size, % EE, % drug release were mainly affected by the concentration of phospholipid and concentrations of edge activators in the formulations. From this study, it was concluded that the optimized formula of Econazole loaded transfersomes, with small particle size, high %EE, and % drug release. F12 batch of Econazole loaded transfersomes formulation was found to be optimized batch having the particle size of 145.25nm and good % entrapment efficiency *i.e.* 80.25 and % drug release 96.65%. Econazole transfersomes may be used as alternative carriers for transdermal drug delivery system because Econazole loaded transfersomes has the ability to overcome the barrier properties of the skin and increase antifungal activity, as compared with the standard drug fluconazole.

Conflict of Interest

None declared

5. REFERENCES

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