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Research Article

FORMULATION, DEVELOPMENT AND EVALUATION OF TRANSFERSOMAL GEL OF AMPHOTERICIN B

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ABSTRACT

Fungal infections are amongst the most commonly encountered diseases affecting the skin. Treatment approaches include both topical and oral antifungal agents. The topical route is generally preferred due to the possible side effects of oral medication. Advances in the field of formulation may soon render outdated conventional products such as creams, ointments and gels. Several carrier systems loaded with antifungal drugs have demonstrated promising results in the treatment of skin fungal infections. The aim of the present study was to investigate the potential of transfersomal gel formulations for transdermal delivery of amphotericin B and to evaluate the effect of lipid concentration, ethanol concentration, drug concentration and stirrer time. Characterization of transfersomes was performed by vesicle size, surface charge, entrapment efficiency and stability study. Characterization of transfersomes containing gel performed by the measurement of viscosity, pH measurements, drug content, extrudability study, spreadability and in vitro drug diffusion study. It was found that viscosity of prepared gel TG-12 was 3350cps, % assay was 99.45±0.45, extrudability was 147g and spreadibility (g.cm/sec) was found that 13.25(g.cm/sec) respectively. *In vitro* drug release from transfersomes gel was carried out using Franz diffusion cell method and found 92.23% in 12 hr. In first 30 min it was 23.36 % drug release which slightly high. It was due to the release of free drug present in bag after leaching from transfersomes. Drug release from transferosomal gel formulation was found in very sustained and controlled manner. The prepared gel containing amphotericin B-loaded transfersomal formulation was optimized and can be use for topical preparation for its antifungal affect. The results were obtained which showed that transfersomal gel was a promising candidate for transdermal delivery with targeted and prolonged release of a drug. It also enhances skin permeation of many drugs.

Keywords: Fungal Infections, Transfersomal Gel, Amphotericin B, Franz Diffusion Cell

1. INTRODUCTION

Fungi are parasitic microorganisms which can affect the skin and mucous membrane along with generation of systemic infections of various internal organs [1]. Fungal infections of skin or mucous membrane, in majority, promote visits of victims to dermatologists [2]. It has been reported that 20%-25% of human population show presence of skin fungal infections [3]. Incidences of occurrence of skin fungal infection are very high in immunocompromised patients [4]. Skin fungal infections are categorized into superficial, cutaneous and subcutaneous depending upon the level of tissue invasion [5]. When attack of invading fungi is limited to outermost skin layers only then generated infection is called superficial fungal infection. Tinea versicolor, white piedra and tinea nigra are examples of superficial fungal infections.

Superficial fungal infection leads to increase in the skin pH along with mild scaling, redness and inflammation at the invading site. The barrier nature of skin becomes poor in such a state [6]. Invasion of parasitic fungus into deeper epidermal skin layer develop cutaneous fungal infection. This infection is also known as dermatomycoses and it may have involvement of skin appendages like nails and hairs [7]. Dermatomycoses can also instigate cellular immune response developing pathological variations in patients [8]. Various fungi generating dermatomycoses come under three genera, namely *Epi-dermophyton*, *Trichophyton* and *Microsporum*. Tinea faciei, tinea barbae, tinea capitis and tinea manuum are the examples of cutaneous fungal infections [9]. Furthermore, extension of fungal infection to dermal or subcutaneous region results subcutaneous fungal infection. It is caused by fungi namely *Sporothrix schenckii*

and *Candida albicans* [10]. This fungal infection is characterized by either ulcerated or infiltrated nodular lesions in the infected areas [11]. Maduramycosis and chro momycosis are other examples of subcutaneous fungal infections [12]. Poor skin penetration of hydrophilic antifungal drugs and high dosing frequency of conventional antifungal formulations reduce their effectiveness against skin fungal pathogens [13]. Therefore, several nanocarrier systems have been investigated by pharmaceutical scientists to fulfil these criteria and considerations for topical delivery of antifungal drugs [14]. Transfersomes are flexible or deformable vesicles and hence also called as elastic vesicles. Gregor Cevc in 1991 introduced the concept and term of elastic vesicles. Since then, extensive work is going on worldwide on these elastic vesicles under different titles like flexible vesicles, ethosomes, etc. Transfersome is derived from the Latin word "transferre", meaning "to carry across", and the Greek word "soma", meaning "body". A transfersome carrier is an artificial vesicle that resembles the natural cell vesicle. Thus it is suitable for both targeted and controlled drug delivery. Functionally, it may be described as lipid droplet with such deformability that permits its easy penetration through the pores much smaller than the droplet size. Transfersome is a highly adaptable and stress-responsive complex aggregate. On topical application, the carrier search and exploits hydrophilic pathways i.e. 'pores' in the skin, which it opens wide enough to permit it to pass through with its drug cargo, deforming itself to accomplish this without losing its vesicular integrity. The vesicle is both self-regulating and self-optimizing due to its interdependency on local composition and shape of the bilayer. This allows the transfersome to cross different transport barriers efficiently. Transfersome penetrates the stratum corneum either via intracellular route or the transcellular route [15, 16]. Amphotericin B (AmB) is the drug of choice for the treatment of systemic fungal infections [17] and is also widely used for the treatment for visceral and mucocutaneous leishmaniasis [18]. However, severe side effects such as nausea, fever and chills and nephrotoxicity accompany the use of AmB [19, 20]. AmB is insoluble in water and therefore, the drug is administered as deoxycholate micelles (Fungizone®) or as liposomal formulations. Clinically used liposomal AmB such as AmBisome®, Amphotec®, and Abelcet® are considered less toxic [21, 22]. However, the instability of liposomes, its high cost of production and the necessity for continuous intravenous infusions prevent their widespread use. AmB can't be absorbed through the skin owing to bulky structure [23]. Transfersome has unique feature to provide improved permeation through skin due to its physico-structural properties. Liposomal as well as niosomal systems, are not suitable for transdermal delivery, because of their poor skin permeability, breaking of vesicles, leakage of drug, aggregation, and fusion of vesicles [24, 25]. To address above mentioned problems, a new type of carrier system called transfersome has recently been introduced, which is capable of delivering low as well as high molecular weight drugs across the skin.

2. MATERIAL AND METHODS

Amphotericin B 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.

2.1. Determination of λ_{max} of AmB

Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.4 pH buffer solution in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.4 pH buffer solution. Prepare suitable dilution to make it to a concentration range of 5-25µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). A graph of concentration Vs absorbance was plotted.

2.2. Preparation of AmB-Loaded Transfersomes

Soya PC (0.5, 1.0, 1.5, 2.0% w/v) was dissolved in ethanol (5-25% v/v) and heated up to 30±1°C in a water bath in a closed vessel [26]. Distilled water or drug solution in distilled water (1% w/v solution), which is previously heated up to 30±1°C, was added slowly in a fine stream to the above ethanolic lipid solution with continuous mixing using a magnetic stirrer at 900 rpm. Mixing was continued for another 5 minutes and finally, the vesicular dispersions resulted was left to cool at room temperature (25±1°C) for 45 minutes.

2.3. Optimization of Transfersomes Formulation

Transfersomes formulation optimized based on the evaluation of mentioned strategy procedure resting on

the source of average vesicle size and (%) entrapment efficiency (EE). In the transfersomal formulation, the ratio of lipid was optimized by taking their different ratio such as 0.5, 1.0, 1.5, and 2.0% w/v ratio and all other parameters were kept remain constant. the ethanol content was optimized by taking their different quantity such as 5, 10, 15, and 20 and all other parameters were kept remain constant. Drug concentration optimized by taking different concentration of drug such as 1, 1.5, and 2.0% w/v and prepared their formulation and all other parameters such as Soya PC, stirrer time kept remain constant. Stirrer time was optimized by stirring the formulation for different time, i.e., 5, 10, and 15 min.

2.4. Characterization of AmB-loaded Transfersomes

2.4.1. Microscopic observation of prepared transferosomes

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared transferosomes formulation.

2.4.2. 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).

2.4.3. Zeta potential

The zeta potential 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.

2.4.4. Entrapment efficiency

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the drug loaded transfersomes dispersion was placed in the Ependorf tubes and centrifuged at 17000 rpm for 30 min. The transfersomes along with encapsulated drug were separated at the bottom of the tubes. Plain transfersomes without drug was used as blank sample and centrifuged in the same manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 284 nm.

2.4.5. Stability studies

Stability study was done 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 months. The formulation subjected for stability study was put away in borosilicate compartment to maintain a strategic distance from any interface among the formulation and glass of container. The formulations were investigated for any physical changes and drug content.

2.5. Preparation of gel base carbopol

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 6.5. Transferosomal preparation corresponding to 2% w/w of amphotericin B was incorporated into the gel base to get the desired concentration of drug in gel base.

2.6. Characterization of transfersomes containing gel

2.6.1. 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 10 rpm.

2.6.2. pH measurements

The pH of selected optimized formulations was established with the help of digital pH meter. The pH meter was calibrated with the help of buffer solution of pH 4, pH 7 and pH 9. After calibration, the electrode was dipped into the vesicles. Then, pH of selected formulation was measured and readings shown on display were noted.

2.6.3. Drug content

Accurately weighed 100 mg of topical transferosomal gel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered by means of Whatman filter paper No. 1. Then, 1.0 ml of filtered solution was engaged in 10 ml capacity of volumetric flask; moreover, volume was ready up to 10 ml by means of methanol. This solution was analyzed using UV spectrophotometer at λmax 284 nm.

2.6.4. Extrudability study

Extrudability was determined on the amount of the gel extruded as of collapsible tube on appliance 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.

2.6.5. Spreadability

Spreadability 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 wooden block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadability, 2-5 gm of gel placed between two slides and gradually weight was increased by adding it on the weight pan and time required with the top plate to face the distance of 10 cm on adding 80 g of weight was noted. Good spreadability shows lesser time to spread. It is determine by formula given below [27].

$$s = \frac{m * l}{t}$$

Where, S=Spreadability (gcm/sec), m = weight tied to the upper slide (20 grams),
l= length of glass slide (6cms), t = time taken is seconds.

2.6.6. In vitro drug diffusion study

The in vitro diffusion study about is conveyed by utilizing Franz diffusion cell. Egg membrane is taken as semi penetrable membrane for diffusion [28]. The Franz diffusion cell has receptor compartment with an effective volume roughly 60 ml and compelling surface area of permeation 3.14sq.cm. The egg membrane is placed between the donor and the receptor compartment. A $2cm^2$ size patch taken and weighed then set on one face of membrane confronting donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is encompassed through water casing to keep up the temperature at 32±0.5°C. Warmth is furnished utilizing a thermostatic hot plate with a magnetic stirrer. The receptor liquid is mixed by Teflon covered magnetic bead which is put in the diffusion cell. Amid each testing interim, samples are pulled back and replaced by equivalent volumes of fresh receptor liquid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of drug 284 nm.

3. RESULTS AND DISCUSSION

The absorption maxima of AmB were determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer (Labindia UV 3000+) using concentration range of 5-25µg/ml AmB in pH 7.4 phosphate buffers. Figure1 AmB showed a linear relationship with correlation coefficient of 0.9998 in the concentration range of 5-25µg/ml in phosphate buffer pH 7.4. Melting point of drug was found 167-169°C while it was 170°C reported in standard monograph. All the data of preformulation study were found similar as given in standard monograph which confirmed that the drug was authenticated and pure in form and it could be used for formulation development of AmB-loaded transfersomes.

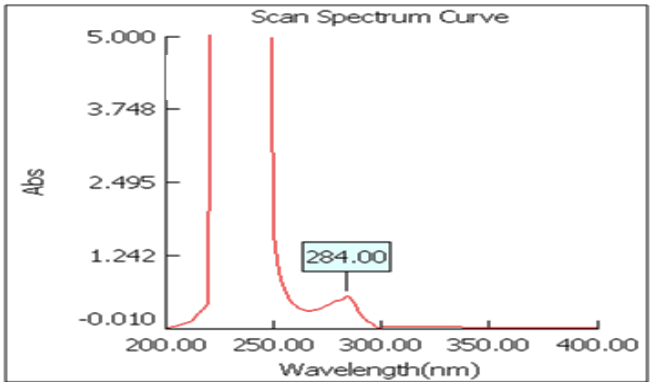

Fig.1: Wavelength maxima of AmB in phosphate buffer pH 7.4

Table 1: Optimization of lipid concentration

F. code	Soya PC (% w/v)	Ethanol	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
F1	0.5	10	1.0	325.32	69.98
F2	**1.0**	**10**	**1.0**	**275.56**	**72.56**
F3	1.5	10	1.0	245.56	65.23
F4	2.0	10	1.0	233.23	48.89

Optimization of the transfersomes to generate the formulation code was done using the strategy as reflected in Table 1 optimization of lipid concentration, Table 2 optimization of ethanol concentration, Table 3 optimization of drug concentration and Table 4 optimization of stirrer time. It was observed that the

vesicles dimension of transfersomes was increased with raising the concentration of phosphatidylcholine and ethanol. There was no noteworthy difference observed in average vesicle size with increasing the drug concentration, but with increase in the stirrer time the size of vesicle decreased from 145.45 to 125.65 after 15 min of stirring. Considering the EE, it was observed that the percent drug entrapment decreased with escalating the concentration of ethanol and on escalating the time of stirring. It is due to the leaching out the drug from vesicles on increasing the mechanical force by stirrer and size reduction of transfersomes on increasing the concentration of ethanol.

Table 2: Optimization of Ethanol Concentration

F. code	Soya PC (% w/v)	Ethanol	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
F5	1.0	5	1.0	289.85	68.89
F6	**1.0**	**10**	**1.0**	**210.23**	**73.32**
F7	1.0	15	1.0	263.32	63.32
F8	1.0	20	1.0	252.12	64.47

Table 3: Optimization of Drug Concentration

F. code	Soya PC (% w/v)	Drug (% w/v)	Ethanol (ml)	Average vesicle size (nm)	% Entrapment efficiency
F9	**1.0**	**1.0**	**10**	**245.56**	**78.25**
F10	1.0	1.5	10	265.23	65.23
F11	1.0	2.0	10	230.45	45.58

Table 4: Optimization of Stirrer Time

F. code	Soya PC: (% w/v)	Drug (% w/v)	Stirrer time (min)	Average vesicle size (nm)	% Entrapment efficiency
F12	**1.0**	**1.0**	**5**	**133.12**	**78.25**
F13	1.0	1.0	10	145.45	63.32
F14	1.0	1.0	15	125.65	60.23

Table 5: Characterization of Optimized Formulation of Transfersomes

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temperature	4.0±0.2°C	25-28±2°C	4.0±0.2°C	25-28±2°C	4.0 ±0.2°C	25-28±2°C
Average particle size (nm)	133.12	145.25	130.14	205.45	142.23	236.65
% EE	78.25	69.98	70.23	55.52	69.32	50.32
Physical Appearance	Normal	Turbid	Normal	High turbid	Normal	High turbid

It was clearly shown when formulation was stirred for 5, 10, and 15 min then the % EE was 78.25, 63.32 and 60.23 is selected as optimized time for stirrer because it provided the required size of vesicle 133.12 nm and good % EE, i.e., 78.25.

The resulted formulation code F-12 was considered as the optimized formulation. The average vesicle size of optimized formulation (F-12) observed as 133.12 nm, zeta potential observed as -32.4mV and % EE was found as 78.25%. Stability study was performed on optimized formulation (F-12) and its characterization depicted in Table 5. Stability study data revealed that the optimized formulation (F-12) was stable after 3 months of storage at 4.0°C±0.2°C while at 25-28±2°C, the formulation was found unstable. Stability of formulation was observed on the basis of % drug remain, average vesicles size and physical appearance. Prepared gel of transfersomes loaded with AmB (TG-12) was prepared

and evaluated for viscosity, pH, % drug content, extrudability, spreadability and drug release study. It was found that viscosity of prepared gel TG-12 was 3350cps, % assay was 99.45±0.45, extrudability was 147g and spreadibility (g.cm/sec) was found that 13.25(g.cm/sec) respectively. *In vitro* drug release (Table 6 & Figure 2) from transfersomes gel was carried out using Franz diffusion cell method and found 92.23% in 12 hr. In first 30 min it was 23.36 % drug release which slightly high. It was due to the release of free drug present in bag after leaching from transfersomes. Drug release from transferosomal gel formulation was found in very sustained and controlled manner.

Table 6: *In vitro* drug release study of prepared gel formulation

S. No.	Time (hr)	% Cumulative Drug Release
1	0.5	23.36
2	1	39.98
3	2	46.65
4	4	55.52
5	6	69.98
6	8	73.32
7	12	92.23

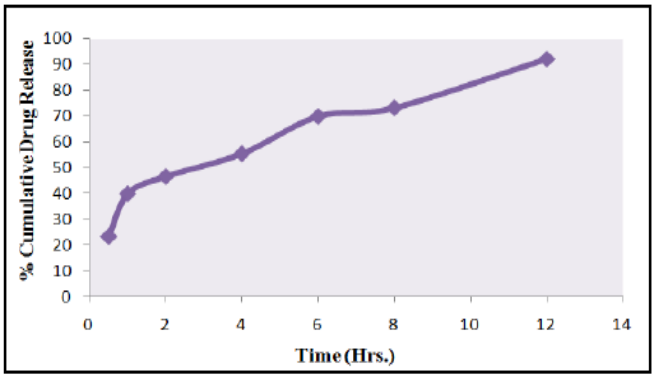

Fig. 2: *In vitro* drug release of gel based transferosomal gel

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. Optimized formulation (F-12) of transfersomes resulted in average vesicle size as 133.12 nm, zeta potential as -32.4mV and % EE as 78.25% and stability study data revealed that the optimized formulation was stable after 3 months of storage at 4.0°±0.2°C. Prepared gel of optimized formulation viscosity was 3350cps, % drug content was 99.45±0.45, extrudability was 147g, spreadability (g.cm/sec) was 13.25 (g.cm/sec) and in vitro drug release found as 92.23 % in 12 h, respectively. It can be concluded that prepared gel containing AmB-loaded transfersomal formulation was optimized and can be of use for topical preparation for its antifungal effect.

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