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Development and characterization of transfersomes for effective treatment of fungal disease (Itraconazole)

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ABSTRACT: Occurrence of skin fungal infections is increasing nowadays and their presence is more prominent in patients suffering from immune compromised diseases like AIDS. Skin fungal infections are a major cause of visits by patients to dermatology clinics. 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 Advances in the field of formulation may soon render outdated conventional products such as creams, ointments and gels.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 Itraconazole and to evaluate the effect of lipid concentration, ethanol concentration, drug concentration and stirrer time. Characterization of transfersomes 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 was 3240cps, % assay was 98.56±0.23, extrudability was 145g and spreadability was found that 9.85 (g.cm/sec) respectively. In vitro drug release from transfersomes was carried out using Franz diffusion cell method and found 92.23±0.21% in 12 hr. In first 30 min it was 12.25±0.32% 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 formulation was found in very sustained and controlled manner. The prepared gel containing Itraconazole-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, Itraconazole, Franz diffusion cell

I. INTRODUCTION

Transfersomes, alias ultradeformable nano-liposomes are obtained by adding surfactants (such as sodium cholate, etc.) in phospholipid bilayers of conventional liposomes. Besides advantages of the good affinity with skin, innocuity and safety, the priorities associated with transfersomes are their high deformability and high skin penetration. The drugs transfer across the skin from the epidermis into the dermis and finally into lymphatic vessels and blood vessels, and possess a therapeutic effect. A research report on transfersomes system is first proposed by professor Cevc from Technical University of Munich indicates that transfersomes membrane has high deformability and its composition is stable via the skin. It is reported by Zellmer that there are complete transfersomes in blood circulation, which have extraordinary superiority in transdermal administration [1-8]. Itraconazole is a lipophilic triazole-derivative antifungal agent with an extremely broad spectrum, used in treating superficial mycotic infection in clinical trials. Currently, capsule and injection are the main dosage forms of products sold in the market. However, the absorption of itraconazole capsules is irregular, the blood concentration fluctuates widely and plasma protein binding rate is up to 99%. In addition, long-term and excessive administration of itraconazole injection leads to drug accumulation and hepatotoxicity [9, 10]. Itraconazole, which is made into transdermal patch with transfersomes as carriers, transfers across the skin from the epidermis into the dermis and finally into lymphatic vessels and blood vessels. Steady-state concentration keeps for a long time and peak and valley concentration are avoided. Moreover, both side effect and the times of administration are reduced. Subsequently, the patients compliance is enhanced to possess the better therapeutic effect of itraconazole. Itraconazole can be easily dissolved

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in dichloromethane and chloroform but water, methanol and ethanol. Itraconazole has a molecular weight of 705.6 with the partition coefficient in noctanol/water as (log P = 5.66) and the solubility as (<1µg/ ml) [11]. Itraconazole is regarded as a lipophilic model drug to investigate the preparation technology to obtain transfersomes with stable quality to provide methodology reference for studies on transfersomes drug-loading system and diadermic preparation of other lipophilic drugs.

II. MATERIALS AND METHODS

Materials

Itraconazole was obtained as a gift sample from Macleods Pharmaceuticals, Mumbai. 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.

Methods

Determination of λ_{max} of Itraconazole

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.

Preparation of Itraconazole loaded transfersomes

Soya PC (0.5, 1.0, 1.5, 2.0% w/v) was dissolved in ethanol (5-20% v/v) and heated up to 30±1°C in a water bath in a closed vessel. 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 [12].

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.

Characterization of Itraconazole-loaded Transfersomes

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.

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

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.

Entrapment efficiency

One ml of Transfersomes suspension was centrifuged at 15.000 rpm for 1 h to allow the separation the entrapped drug from the un-entrapped drug. After centrifugation, the centrifuge then analyzed spectrophotometrically at 264nm using a UV spectrophotometer (Labindia 3000+). The EE% of Itraconazole in the prepared Transfersomes was calculated applying the following equation:

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$$\% \text{ Entrapment Efficiency } = \frac{\text{Terotical drug content} - \text{Practical drug content}}{\text{Therotical drug content}} \times 100$$

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 1% w/w of Itraconazole was incorporated into the gel base to get the desired concentration of drug in gel base.

Characterization of Transfersomes containing Gel

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.

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.

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} 264nm.

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 determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

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 wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadability, placing 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 spreadability show lesser time to spread [13].

$$Spreadibility\,(g.cm/\sec) = \frac{Weight\ tide\ to\ Upper\ Slide \times Lenth\ moved\ on\ the\ glass\ slide}{Time\ taken\ to\ slide}$$

In vitro drug diffusion study

The In-vitro diffusion study is carried by using Franz Diffusion Cell. Egg membrane is taken as semi permeable membrane for diffusion [14]. The Franz diffusion cell has receptor compartment with an effective volume approximately 60 ml and effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32 ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of drug 264nm.

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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.

III. RESULTS AND DISCUSSIONS

The absorption maxima of itraconazole were determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer (Labindia UV 3000+) using concentration range of 10-50µg/ml itraconazole in 7.4phosphate buffers Figure1. Itraconazole showed a linear relationship with correlation coefficient of 0.999 in the concentration range of 10-50µg/ml in phosphate buffer pH 7.4. 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 itraconazole-loaded transfersomes.

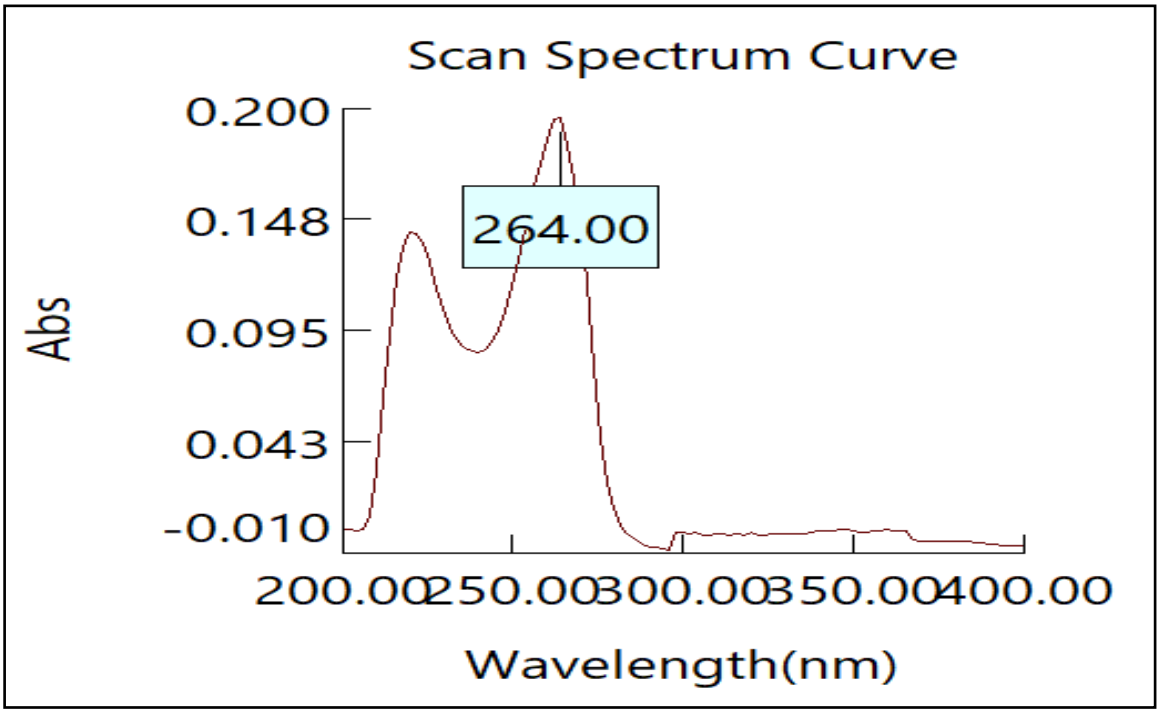

Figure 1 Wavelength maxima of itraconazole in phosphate buffer pH 7.4

Optimization of the transfersomes to generate the formulation code was done using the strategy as reflected in Table 1 optimization of lipid concentration, optimization of ethanol concentration, optimization of drug concentration and 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 155.23 to 115.56 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. It was clearly shown when formulation was stirred for 5, 10, and 15 min then the % EE was 52.23, 55.65, and 73.32 minis selected as optimized time for stirrer because it provided the required size of vesicle 115.56 nm and good % EE, i.e., 73.32. The resulted formulation code F-14 was considered as the optimized formulation. The average vesicle size of optimized formulation (F-14) observed as 115.56 nm, zeta potential observed as -36.45mV and %EE was found as 73.32%.

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Stability study was performed on optimized formulation (F-14) and its characterization depicted in Table 2. Stability study data revealed that the optimized formulation (F-14) 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 was prepared and evaluate for viscosity, % assay, extrudability, spreadability and drug release study. It was found that viscosity of prepared gel was 3240cps, % assay was 98.56±0.23, Extrudability was 145g and Spreadability (g.cm/sec) was found that 9.85 (g.cm/sec) respectively Table 3. In vitro Drug Release: In vitro drug release from Transfersomes was carried out using Franz diffusion cell method and found 92.23±0.21% in 12 hr. In first 30 min it was 12.25±0.32% 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 formulation was found in very sustained and controlled manner.

Table 1 Optimization of transfersomes formulation

Formulation code	Soya PC (% w/v)	Ethanol	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
Optimization of lipid concentration					
F1	0.5	10	1.0	325.56	56.65
F2	**1.0**	**10**	**1.0**	**285.95**	**73.32**
F3	1.5	10	1.0	236.68	45.65
F4	2.0	10	1.0	345.44	69.98
Optimization of ethanol concentration					
F5	1.0	5	1.0	300.21	65.56
F6	**1.0**	**10**	**1.0**	**252.36**	**76.65**
F7	1.0	15	1.0	296.65	63.32
F8	1.0	20	1.0	315.56	59.98
Optimization of ethanol concentration					
F9	**1.0**	**1.0**	**10**	**198.85**	**75.65**
F10	1.0	1.5	10	235.23	65.45
F11	1.0	2.0	10	165.74	43.32
Optimization of drug concentration					
F12	1.0	1.0	5	155.23	52.23
F13	1.0	1.0	10	142.45	55.65
F14	**1.0**	**1.0**	**15**	**115.56**	**73.32**

Table 2 Characterization of optimized formulation of Transfersomes formulation

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 Vesicle size (nm)	120.23	165.58	125.45	240.36	127.32	295.58
% EE	73.12	63.32	71.54	52.21	71.04	48.89
Physical Appearance	Normal	Turbid	Normal	High turbid	Normal	High turbid

Table 3 Results of Characterization of gel based formulation containing Itraconazole

Gel 1%	Viscosity (cps)	Assay (%)	Extrudability (g)	Spreadability (g.cm/sec)
	3240	98.56±0.23	145	9.85

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IV. 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-14) of transfersomes resulted in average vesicle size as 115.56 nm, zeta potential as -36.45mV and % EE as 73.32% 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 3240cps, % drug content was 98.56±0.23, extrudability was 145g, spreadability (g.cm/sec) was 9.85 (g.cm/sec) and in vitro drug release found as 92.23±0.21% in 12 h, respectively. It can be concluded that prepared gel containing Itraconazole-loaded transfersomal formulation was optimized and can be of use for topical preparation for its antifungal effect.

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