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FORMULATION AND EVALUATION OF ITRACONAZOLE NIOSOMAL GEL FOR TOPICAL APPLICATION

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Abstract:

Itraconazole is a broad spectrum Imidazole derivative useful in the treatment of superfacial and systemic fungal infection. The present study was to formulate and evaluate the Itraconazole niosomal gel using surfactant span 40, 60 and tween 60 for the preparation of niosomes. The main objective of the study was to enhance the antifungal activity of the formulation. Itraconazole niosomes were prepared by thin film hydration method using span 40, 60 and tween 60 (as non-ionic surfactant) and cholesterol (as stable vesicle forming agent). Niosomes were prepared using different ratio of drug: surfactant: cholesterol (1:1:0.2, 1:1.5:0.3, 1:2:0.4). The niosomal dispersion was evaluated for vesicle size, surface morphology, percent entrapment efficiency, drug content and in vitro drug release. The entrapment efficiency and drug content were calculated at 262 nm using UV spectrophotometer. The entrapment efficiency was found to be 71.2%, 62.2% and 59.2% for the formulations F5, F7 and F6. Itraconazole niosomal gel was prepared using Carbopol 940, glycerol, triethanolamine and distilled water. Evaluation of niosomal gel was determined by physical appearance, pH, viscosity, drug content, entrapment efficiency and In-vitro permeation studies. The percentage of the drug release from the niosomal gel was found to be 98.87% for FG-2 after 24 hrs. The present study demonstrates prolongation of drug release, an increase in amount of drug retention into skin and improved permeation across the skin after encapsulation of Itraconazole into niosomal topical gel.

Keywords: *Itraconazole, Niosomes, Fungal infection, Thin film hydration method, Carbopol 940.*

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INTRODUCTION:

Drug delivery systems (DDSs) are a new advanced system of drug delivery now a days [1]. It consists of nano particles liquid crystal vesicles which are biocompatible and produces higher efficacy by helping reduction in development of new drugs [2]. There is lot of new advances in biotechnology that helps to introduce new pharmaceutical agents such as proteins, peptides, oligonucleotides and plasmids. DDSs also help to overcome to side effects that are associated with the drugs [3]. Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkylpolyglycerol ether class and cholesterol with subsequent hydration in aqueous media. The basic process of preparation is the same i.e. hydration by aqueous phase of the lipid phase which may be either a pure surfactant or a mixture of surfactant with cholesterol [4]. After preparing niosomal dispersion, unentrapped drug is separated by dialysis centrifugation or gel filtration. A method of in-vitro release rate study includes the use of dialysis tubing. Niosomes are promising vehicle for drug delivery and being non-ionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. Niosomes are unilamellar or multilamellar [5]. Itraconazole (ITZ) is an orally active triazole antimycotic agent, which is active against a broad spectrum of fungal species [6]. ITZ has been classified as a biopharmaceutics classification scheme (BCS) Class II drug. It is also very lipophilic with an octanol/water log partition coefficient of 5.66 at a pH of 8.1. It is practically insoluble in water (~4ng/ml) [7-9]. Therefore, the bioavailability of unformulated ITZ is extremely low. Itraconazole has low solubility and low permeation. By incorporation of Itraconazole in small niosomes, the drug can be targeted directly to the site of action, thus enhancing its therapeutic efficacy.

MATERIALS AND METHODS:

Materials:

ITZ was kindly donated by Mylan Laboratories Limited, Hyderabad, India. Cholesterol, Span, 40, 60 and Tween 60 were obtained from Central drug house Pvt. Ltd., New Delhi. Carbopol 940 was obtained from Qualikems Fine Chem. Pvt. Ltd., Barodra. Chloroform was obtained from Thermo Fischer Scientific India Pvt. Ltd., Mumbai. Methanol was obtained from Merck Specialities Ltd.

Formulation of Itraconazole niosomes:

The niosomal formulations were prepared by thin film hydration technique. Accurately weighed quantities of drug (100mg), non-ionic surfactant (Span 40, 60, Tween 60) and cholesterol were dissolved in sufficient quantity of solvent mixture (Chloroform: Methanol 2:1) to give a clear solution. The resulting solution is poured into a 1000 ml rotary flask and evaporated under vacuum (20-25mm Hg) at 60°±2°C with the rotation speed of 100 rpm to form a uniform thin dry film. The rotary flask was removed from the bath and allowed to return to room temperature. The thin film formed was hydrated with 20 ml of distilled water while rotating the flask at 50 rpm (gentle agitation) at a temperature 60°±2°C. The resulting niosomal suspension was stored in a tightly closed container in a refrigerator [10].

Table 1Formulation code of niosomes

Formulation Code	Non-Ionic Surfactant	Drug: Surfactant: Cholesterol (m moles)
F1	Tween 60	1:1:0.2
F2	Tween 60	1:1.5:0.3
F3	Tween 60	1:2:0.4
F4	Span 40	1:1:0.2
F5	Span 40	1:1.5:0.3
F6	Span 40	1:2:0.4
F7	Span 60	1:1:0.2
F8	Span 60	1:1.5:0.3
F9	Span 60	1:2:0.4

In the Drug: Surfactant: Cholesterol ratio, 1 stands for 25 µmol.

Formulation of niosome entrapped itraconazole gel:

The promising niosomal suspension, (formulation of niosomes prepared using the optimized ratio of surfactants) containing Itraconazole equivalent to 2% w/w was incorporated into the gel base composed of Carbopol 940 (0.5, 1 and 1.5 %%), Glycerol (10%), Triethanolamine (q.s.) and distilled water up to 15gm [10].

Characterization of niosomes:

Particle size analysis:

The Niosomal suspension was diluted, filled in a cuvette using suitable blank and the average vesicle size of the Niosomes was determined using Malvern zeta sizer [11].

Drug content analysis:

The amount of drug in the formulation was determined by lysing the niosomes using 50% n-propanol. 1 ml of the niosomal preparation was pipettedout, sufficient quantity of 50% n-propanol was added and shaken well for the complete lysis of the vesicles. After suitable dilution with the phosphate buffered saline of pH 7.4 containing 10% Methanol, the absorbance of the solution was measured at 262 nm in the UV- Visible Spectrophotometer. The excipients mixture without the drug treated in the similar manner as the niosomal suspension was used as blank. The drug content was calculated [12].

Estimation of entrapment efficiency:

The entrapment efficiency of the formulations was determined by centrifuging 1 ml of the suspension diluted to 10 ml with distilled water at 15,000 rpm for 60 minutes at 4°C using a high speed cooling centrifuge in order to separate niosomes from unentrapped drug. The free drug concentration in the supernatant was determined at 262nm using UV-Visible Spectrophotometer after suitable dilution. The percentage of drug entrapment in niosomes was calculated using the following formula,

% drug entrapment = (Total drug- Drug in supernatant liquid) X 100 Total drug

***In- Vitro* release study:**

In- vitro drug release pattern was studied using dialysis membrane. The niosomalpreparation after separation of unentrapped drug was placed in an open ended glass tube, one end of which was tied with the dialysis membrane. This acted as the donor compartment. Then the open ended tube was placed in a beaker containing 100 ml phosphate buffered saline pH 7.4, which acted as receptor compartment. The temperature of the receptor medium was maintained at 37°±2°C and the medium was agitated at a speed of 100 rpm using a magnetic stirrer. 5ml of the samples were collected at a predetermined time and replenished immediately with the same volume of fresh buffer PBS pH 7.4. The sink condition was maintained throughout the experiment. The collected samples were analyzed spectrophotometrically at 262nm using UV-Visible spectrophotometer.

Scanning electron microscopy:

The sizes of the vesicles were measured by scanning electron microscope (HITACHI S- 150). A small amount of sample of niosomes suspension was taken in cover slip on the specimen stub. It was coated with carbon and then with gold vapour using Hitachi vacuum evaporator, model HITACHI S 5 GB. The samples were examined under scanning electron microscope, which is operated at 15 kilovolts and then photographed.

Evaluation of niosomal gel:

Physical appearance:

The prepared gel was examined for clarity, color, homogeneity and the presence of foreign particles.

Ph:

2.5gm of gel was accurately weighed and dispersed in 25 ml of distilled water. The pH of the dispersion was measured by using a digital pH meter.

Viscosity measurement:

Viscosity was determined by Brookfield programmable DV III ultra viscometer. In the present study, spindle no. CP 52 with an optimum speed of 0.01 rpm was used to measure the viscosity of the preparation.

Content uniformity:

The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel equivalent to 10mg of the drug in 100 ml volumetric flask and suitable volume of 50% n-propanol for lysis of the vesicles. The volume was made up to 100 ml with methanol. The content was filtered through Whatman filter paper No.41. 5 ml of above solution was taken into a 50ml volumetric flask and volume was made upto mark with methanol. The content of Itraconazole was determined at 262 nm against blank by using the Shimadzu UV/visible spectrophotometer.

Spreadability:

An important criterion for gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to skin. The therapeutic efficacy of a formulation also depends on its spreading value. A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from formulation, placed between, under the application of

a certain load. Lesser the time taken for the separation of two slides, better the spreadability. It is determine by formula given below.

$$\text{Spreadability} = \frac{m.l}{t}$$

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.

Estimation of entrapment efficiency:
The entrapment efficiency of the formulations was determined by centrifuging 0.5 g of the gel equivalent to 10mg of Itraconazole diluted to 10 ml with distilled water at 15,000 rpm for 60 minutes at 4°C using a high speed cooling centrifuge in order to separate niosomes from unentrapped drug. The free drug concentration in the supernatant was determined at 262 nm using UV-Visible Spectrophotometer after suitable dilution. The percentage of drug entrapment in niosomes was calculated using the following formula,

%drug entrapment= (Total drug- Drug in supernatant liquid)/ Total drug X 100

***In-vitro* drug diffusion study:**
In- vitro drug diffusion study was studied using dialysis membrane. The niosomalgel equivalent to 10mg of the drug was placed in an open ended glass tube, one end of which was tied with the dialysis membrane. This acted as the donor compartment. Then the open ended tube was placed in a beaker containing 100 ml phosphate buffered saline pH 7.4, which acted as receptor compartment. The temperature of the receptor medium was maintained at 37°±2°C and the medium was agitated at a speed of 100 rpm using a magnetic stirrer.5ml of the samples were collected at a predetermined time and replenished immediately with the same volume of fresh buffer PBS pH 7.4. The sink condition was maintained throughout the experiment. The collected samples were analyzed spectrophotometrically at 262nm using UV- Visible spectrophotometer.

Stability studies:
The stability studies of the optimized niosomal formulations were performed at different conditions of temperature and the effect on physical characterisitics, entrapment efficiency and drug content was noted. The niosomal dispersions were kept in the air tight containers and stored at 2-8°C and at room temperature (30±2°C) for 30 days and 2 ml samples were withdrawn every 15 days and at the end of 45 days. The samples were analyzed spectrophotometrically at λmax 262nm after disrupting the vesicles with 50% n-propanol.

RESULTS AND DISCUSSIONS:

The IR spectrum of sample drug shows the peak values which are characteristics of the drug and the graph were shown in Fig. 1. The λmax of itraconazole was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer (Shimadzu-1601, Kroyoto, Japan) using7.4 pH buffer solution as solvent and concentration range of 2-10µg/ml Fig. 2. In the present research work Itraconazole niosomes were prepared using different ratios of drug: surfactant: cholesterol and optimization of process-related variables by the thin film hydration method. The prepared Itraconazole niosomes were evaluated for various parameters like particle size, shape, entrapment efficiency and in vitro drug release. Finally, the promising formulation was selected and then it was incorporated into the gel for topical uses. The result of average vesicle size, % entrapment efficiency and zeta potential of optimized formulation (F5) of niosomes was given in table 2. The result of viscosity (cps), % drug content, % Entrapment efficiency, spreadability (g.cm/sec) and pH of optimized formulation (FG-2) of niosomes gel was given in table 3. The in-vitro diffusion study is carried by using Franz Diffusion Cell. Egg membrane is taken as semi permeable membrane for diffusion. The result of In vitro drug release study of prepared gel formulation was given in table 4. In vitro drug release of plain gel was found to be 78.59 in 6 hrs but niosomal gel gave drug release 98.87 in 24hrs. Gel formulation containing niosomes loaded with Itraconazole showed prolonged action than formulations containing Itraconazole in non-niosomal form. Stability studies of the Itraconazole

optimizedgel formulations (FG2) were carried out by storing at 4°C - 8°C (refrigeration temperature) and 25°C ±2°C (room temperature) for a period of 45 days as per ICH (International Conference on Harmonization) guidelines and result was given in table 5. The entrapment efficiency of the drug in the niosomal gel was estimated immediately after the preparation and after every 15 days for 45 days. The drug leakage from the vesicles was least at 4°C. This may be attributed to phase transition of surfactant and lipid causing leakage of vesicles occurs at higher temperature at storage. Hence, the niosomes can be stored at 4-8°C. The improved stability of niosomes after incorporation into the gel base may be due to prevention of fusion of niosomes. The higher drug skin retention in case of niosomal gel maybe due to creation of reservoir effect of drug in the skin and thereby increasing the drug retention capacity into the skin.

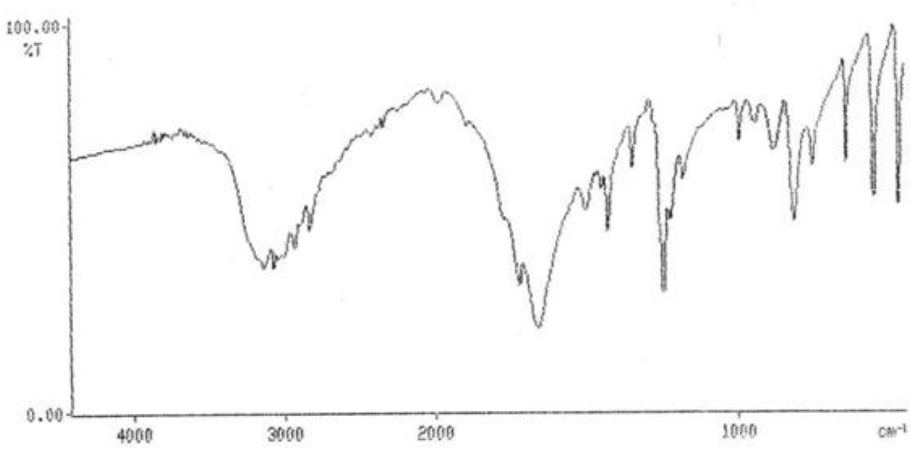

Figure 1 FT- IR spectra of Itraconazole

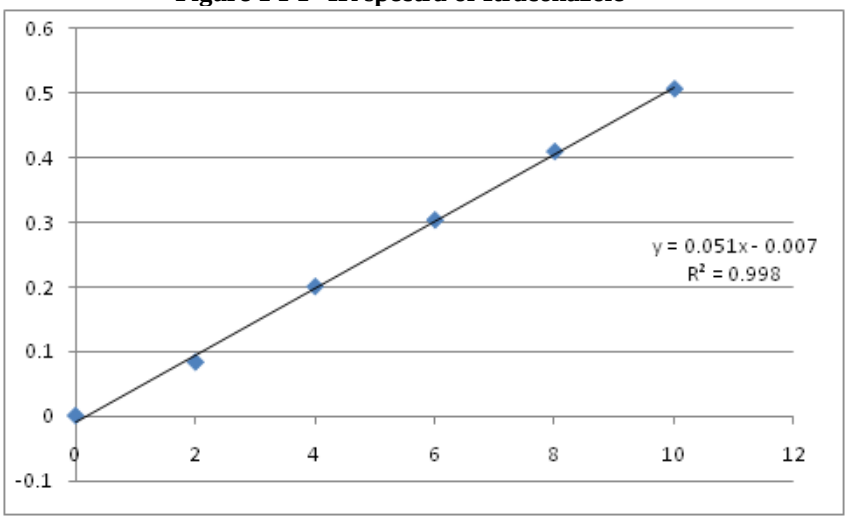

Figure 2 Standard curve of Itraconazole in PBS pH 7.4

Table 2 Characterization of Optimized formulation of Niosomes

Characterization	Average vesicle size (nm)	% Entrapment efficiency	% Drug Content (% W/W)
F5	272.3 nm	71.2	85.54

Table 3 Characterization of gel based formulation of niosomes

Parameters					
F. code	**Viscosity (cps)**	**% Drug content**	**% Entrapment efficiency (%w/w)**	**Spreadibility (g.cm/sec)**	**pH**
FG2	2146	96.56±0.12	69.2	13.22±0.25	7.0±0.12

Table 4 In vitro drug release study of prepared gel formulation (FG2)

Time in (Hrs)	Cumulative % Drug Release (% w/w)				
	Plain Itraconazole Gel	FG1	FG2	FG3	Phytoral
0	0	0	0	0	0
1	5.96	2.08	2.47	2.28	3.45
2	28.78	29.78	28.45	26.45	36.23
3	46.76	45.50	44.65	42.89	44.34
4	48.81	51.36	50.45	49.42	50.34
5	61.85	65.34	64.56	63.23	65.23
6	78.59	68.34	69.65	70.65	72.34
7		71.34	70.43	72.45	73.34
8		76.34	77.45	76.34	76.45
9		78.23	79.54	80.12	80.45
10		80.34	81.45	82.23	82.45
11		81.43	82.44	85.45	86.34
12		84.34	86.67	87.34	88.34
24		85.56	98.87	94.45	95.45

Table 5 Stability study of Itraconazole niosomal gel formulation FG2 at different temperature

Time of storage in days	Temperature of storage			
	(%)Drug Content 4-8^{0}C (refrigeration temperature)	(%)Entrapment efficiency 4-8^{0}C (refrigeration temperature)	(%)Drug Content 25°C ±2°C (room temperature)	(%)Entrapment efficiency 25°C ±2°C (room temperature)
0	97.10	64.8	97.10	64.8
15	96.90	64.5	96.10	64.0
30	96.71	64.2	95.16	63.0
45	96.32	64.0	94.58	62.4

CONCLUSION:

The purpose of this research was to prepare Itraconazole loaded niosomes for controlled release of drug and incorporate it in to topical gel delivery system to reduce the side effects. Thin film hydration technique was employed to produce niosomes using non-ionic surfactants and cholesterol. The results of the FT- IR studies proved that there is no interaction between the drug cholesterol and the non-ionic surfactants. The process related parameters were optimized such as hydration time (60 minutes), sonication time (10minutes), rotational speed of the evaporator flask (initially 100 rpm, later 150 rpm). Cholesterol is used as a membrane additive, acts as a stabilizer as well as fluidity buffer to improve the stability of the vesicles. The formulations were prepared using different non- ionic surfactants by varying the surfactant concentration (Span40, 60 and Tween 60) and keeping the cholesterol concentration fixed. The prolonged release of the drug from the niosome suggests that the frequency of administration and adverse effects significantly thereby improving the patient compliance. The administration of drug as gel type formulation enhances its penetration and release.

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