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Formulation and Evaluation of Itraconazole Niosomal Gel for Topical Application

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ABSTRACT

Niosomes have potential applications in topical drug delivery system. Niosomes play an increasingly important role in drug delivery as they can reduce toxicity and modify pharmacokinetic and bio-availability. Topically applied niosomes can increase the residence time of drugs in the stratum corneum and epidermis, while reducing the systemic absorption of the drug. It can act as drug containing reservoirs and the modification of the vesicular compositions or surface properties can adjust the drug release rate and the affinity for the target site. Itraconazole is a triazole derivative useful in the treatment of number of fungal infections. This includes aspergillosis, blastomycosis, coccidioidomycosis, histoplasmosis, and paracoccidioidomycosis. It may be given by mouth or intravenously. Itraconazole niosomes were prepared by thin film hydration method using span 20, 40, 60 (as non-ionic surfactant) and cholesterol (as stable vesicle forming agent). Niosomes were prepared using different ratio of drug: surfactant: cholesterol (1:1:1, 1:2:1, 1:3:1). 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 57.2%, 73.2% and 61.2% for the formulations ITZ 20-3, ITZ 40-2 and ITZ 60-1. 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 55.67 % for ITZG-2. 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: Niosomes, Itraconazole, Fungal infection, Thin film hydration method, Carbopol 940

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INTRODUCTION

Drug delivery systems using vesicular carriers such as liposomes[1] and niosomes[2] have distinct advantages over conventional dosage forms because the vesicle can act as drug containing reservoirs. Most antifungal drug substances are lipophilic compounds, which are practically insoluble in water[3]. For skin care and the topical treatment of dermatological disease, a wide choice of vehicles ranging from solids to semisolids and liquid preparations is available to clinicians and patients[4]. Topical application of antimicrobial agents is a useful tool for the therapy of skin and soft-tissue infections[4]. A number of strategies to deliver antifungal using nanocarriers are developed to facilitate drug targeting infected cells. Nanosized carriers have been receiving special attention with the aim of minimizing the side effects and improving efficacy of drug therapy. Several nanosized delivery systems have already proved their effectiveness in antifungal therapy[5]. The total therapeutic effect of percutaneous preparations depends not only on the action of the drug itself, but also on other factors related to the structure of the vehicle. Taking into account the peculiarities of fungal parasites, the focus is placed particularly on lipid-based vehicles and earlier studies have shown that results in improved antifungal activity[6]. Many techniques have been aimed to disrupt and weaken the highly organized intercellular lipids in an attempt to enhance drug transport across the intact skin; one of them is the vesicle formulation as skin delivery system[7-10]. Niosomes have been recognized as good vehicles for the topical delivery of drugs[11]. They serve as organic solvent for the solubilization of poorly soluble drugs, for instances corticosteroids; as a result, higher local drug concentrations at the maximum thermodynamic activity can be applied. They may serve as a local depot for the sustained release of dermal active compounds including antibiotics, corticosteroids or retinoic acid. By virtue of penetration of individual phospholipid molecules or nonionic ether surfactants into the lipid layers of the stratum corneum and epidermis, they may serve as penetration enhancer and

facilitate dermal delivery leading to higher localized drug concentrations. They may serve as rate-limiting membrane barrier for the modulation of systemic absorption, that is, they may serve as controlled transdermal delivery systems. Mainly two types of vesicle skin interactions occurs during *in vitro* studies using human skin which may induce various effects on dermal or transdermal drug delivery[12-14] First, the vesicles in contact with stratum corneum aggregate fuse and adhere to the cell surface. It is believed that this interaction leads to a high thermodynamic activity gradient of the drug at the vesicle stratum corneum interface, which is the driving force for penetration of the lipophilic drugs across the stratum corneum. Secondly, this type of interaction involves the ultrastructural changes of the intercellular lipid regions of the stratum corneum and its deeper layers at maximum depth of about 10 μm as revealed by freeze fracture electron microscopy (FFEM) and Small Angle X-ray Scattering (SAXS). Itraconazole (ITZ) is an orally active triazole antimycotic agent, which is active against a broad spectrum of fungal species including *Cryptococcus, Candida, Aspergillus, Blastomyces* and *Histoplasma capsulatum* var. *Capsulatum*[15]. 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[16-18]. It is practically insoluble in water (~4 ng/ml) [19]. Therefore, the bioavailability of unformulated ITZ is extremely low. Itraconazole is a drug of choice for patients with indolent, nonmeningeal infections due to *B. dermatitidis, H. capsulatum, P. brasiliensis,* and *C. immitis.* Approximately half of thepatients with distal subungual onychomycosis respond well to Itraconazole. Itraconazole is often the best choice for the treatment of pseudallescheriasis, an infection not responding to the amphotericin B therapy, as well as cutaneous or extracutaneous sporotrichosis, tinea corporis, and extensive tinea versicolor. Itraconazole is used in the treatment of toenail onychomycosis with terbinafine as one week per month for three months. 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 20, Span 40 and Span 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 20, 40, 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[20].

Table 1 Formulation of Itraconazole niosomes

Formulation Code	Non-Ionic Surfactant	Drug: Surfactant: Cholesterol (m moles)
ITZ20-1	Span 20	1:1:1
ITZ20-2	Span 20	1:2:1
ITZ20-3	Span 20	1:3:1
ITZ40-1	Span 40	1:1:1
ITZ40-2	Span 40	1:2:1
ITZ40-3	Span 40	1:3:1
ITZ60-1	Span 60	1:1:1
ITZ60-2	Span 60	1:2:1
ITZ60-3	Span 60	1:3:1

Optimization of process-related variables

Effect of hydration time

The niosomal formulations containing Span 40 at different ratios and a fixed amount of cholesterol (1:1:1, 1:2:1) were hydrated with 10 ml of distilled water for 30 minutes, 60 minutes and 90 minutes. The vesicle formation and entrapment efficiency of the formulations were calculated by centrifugation method.

Effect of capacity and rotational speed of evaporator flask

The thickness and uniformity of the film depends upon the rotational speed of the evaporator flask. The niosomal formulations were subjected to various speeds i.e. 50 rpm, 100 rpm and 150 rpm. The appearance of the film was checked by visual observation.

Effect of sonication time

The Niosomal formulations containing Span 40 at different ratios and a fixed amount of Cholesterol (1:1, 2:1, 3:1) were subjected to ultrasonic vibration using Ultrasonicator. To study the effect of sonication time, the formulations were subjected to sonication for various time intervals (1 min, 2 min, 3 min, 4 min and 5 min). The entrapment efficiency of the formulations was measured[21, 22].

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[20].

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[23].

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 pipetted out, 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[24].

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[21],

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 niosomal preparation 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[25].

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[26].

Zeta potential analysis

Zeta potential analysis was used to measure the stability of niosome by studying its colloidal property. Aggregation is attributed to the shielding of the vesicle surface charge by ions in solution and thereby reducing the electrostatic repulsion. Vesicle surface charge can be estimated by measurement of particle electrophoretic mobility and is expressed as the Zeta potential. The study was conducted using Malvern Zeta Analyzer[27].

Characterization of niosomes containing 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[20].

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[26],

%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 niosomal gel 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[25].

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[28].

RESULTS AND DISCUSSION

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 λ_{max} 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.

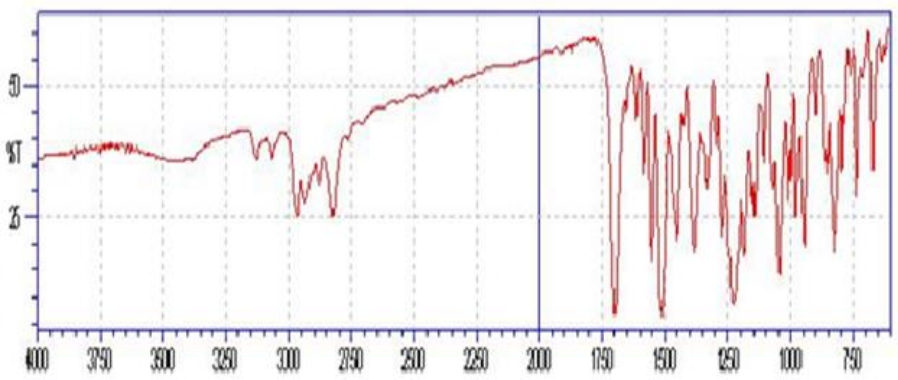

Figure 1 FT- IR spectra of Itraconazole

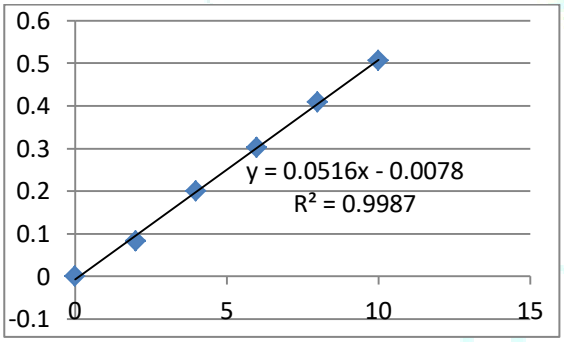

Figure 2 The linear regression analysis for standard curve

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 (ITZ40-2) of niosomes was given in table 2.

Table 2 Characterization of Optimized formulation of Niosomes

Characterization .	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
ITZ40-2	272.3	73.03±2.39	-47.2±0.2

The result of viscosity (cps), % drug content, % Entrapment efficiency, spreadability (g.cm/sec) and pH of optimized formulation (ITZG-2) of niosomes gel was given in table 3.

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**
ITZG-2	2345±2.07	95.56±0.12	68.2	14.22±0.25	7.1±0.15

ITZG * Optimized gel formulation (n=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 optimized gel formulations (ITZG-2) 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.

Table 4 *In vitro* drug release study of prepared gel formulation (ITZG-2)

Time (hr)	% Cumulative Drug Release	
	Plain gel	Niosomal Gel
0	0	0
1	5.96	2.47
2	28.78	28.45
3	46.76	44.65
4	48.81	50.45
5	61.85	64.56
6	78.59	69.65
7		70.43
8		77.45
9		79.54
10		81.45
11		82.44
12		86.67
24		98.87

Table 5 Stability study of Itraconazole niosomal gel formulation ITZG-2 at different temperature

Time of storage in days	Temperature of storage			
	(%)Drug Content 4-8^{0}C (refrigeration temp)	(%)Entrapment efficiency 4-8^{0}C (refrigeration temp)	(%) Drug Content 25°C ±2°C (room temp)	(%)Entrapment efficiency 25°C ±2°C (room tem)
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 (Span 20, 40 and Span 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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