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FORMULATION AND EVALUATION OF ITRACONAZOLE NIOSOMAL GEL

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ABSTRACT

The present study was to formulate and evaluate the Itraconazole niosomal gel using surfactant span 40 and span 60 for the preparation of niosomes. The main objective of the study was to enhance the antifungal activity of the formulation. Itraconazole is a broad spectrum Imidazole derivative useful in the treatment of superfacial and systemic fungal infection. Various surfactants used were span 40 and span 60. Niosomes were prepared by using Rotary Vaccum evaporation method. Niosomes were prepared using different ratio of drug: surfactant: cholesterol (1:1:1, 1:2:1, 1:3:1).Evaluation of the Niosomal gel was done by determination of drug content 52.81-56.12, Entrapment Efficiency 42.20-45.20,Niosomal gel was prepared using Carbopol 940 (1.5%), Glycerol (10%), Triethanolamine (q.s.) and distilled water up to 15ml. Viscosity was determined by Brookfield programmable ultra-viscometer and the ranges 8173 centipoise. The drug content of the Itraconazole niosomal gel was determined at 262 nm against blank by using UV/visible spectrophotometer and found to be 56.12%. The percentage of drug entrapment in niosomal gel was calculated to be 45.20% .The in-vitro drug release study was carried out using phosphate buffer saline pH 7.4 and was found to be 41.18 ± 1.53% for 12 hours. It can be concluded that the gel formulation containing niosomes loaded with Itraconazole showed prolonged action than formulation containing Itraconazole in non-niosomal form and it can be developed successfully to improve the anti-fungal activity.

KEYWORDS: Niosome, Itraconazole, Cholesterol, Span 60/ Span 40, Diethyl Ether, Carbopol 940, Glycerol, Rotary Vacuum Evaporator.

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INTRODUCTION

Drug delivery systems (DDSs) is 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 helps 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 dialkyl polyglycerol 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].

Material: - Itraconazole was a gift sample from Titans pharma, Tahliwal, and Cholesterol was obtained from Central drug house Pvt. Ltd., New Delhi, Span 40 and Span 60 was obtained from Central drug house Pvt. Ltd., New Delhi, Chloroform Thermo Fisher Scientific India Pvt. Ltd., Mumbai, Buffer (pH 7.4) Laboratory preparation, Carbopol 934 was obtained from Hi Media Laboratories, Mumbai.

Method

Preparation of Itraconazole Niosomes by Reverse phase evaporation method

Span-40 and Span-60: Cholesterol at a ratio of 1:1:1, 1:2:1, 1:3:1, by weight and 100mg of Itraconazole were dissolved in 20 ml of Dichloromethane, in a 250 ml

round-bottomed-flask. The flask was fitted onto a rotary flask evaporator and connected to a vacuum pump. The solvent system was evaporated under vacuum at 50^0C using water bath for 10 minutes. The dried surfactant film was hydrated with 50 ml distilled water for 30 minutes 50^0Cusing bath sonicator. The hydrated dispersion was kept in refrigerator for 2 hours for sealing of vesicles**.** The niosomal suspension was formed and stored in refrigerator in closed container

Table 1: Formulation Code of Niosomes containing surfactant Span 40

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

Table 2: Formulation Code of Niosomes containing surfactant Span 60

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

EVALUATION OF ITRACONAZOLE NIOSOMES

Particle size analysis

The average particle size of the niosomes was characterize using Malvern's zeta sizer. The niosomal suspension was diluted, filled in a cuvette using suitable blank.[8]

Drug content analysis

Drug content in the niosomal suspension equivalent to 100mg was determined by lysing the niosomes using n-propanol. 1ml of the lysed niosomal solution was then diluted up to 10ml using 7.4 phosphate buffers. The absorbance of the dilution was then calculated spectrophotometrically at 262nm.[9,10]

Entrapment efficiency

The free drug concentration in the supernatant was determined at 225 nm using UV- Visible Spectrophotometer after 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 so as to separate niosomes from unentrapped drug. The % drug entrapment was calculated from the following formula.[11]

$$\% \text{ drug entrapment} = \frac{(\text{Total drug} - \text{Drug in supernatant liquid}) \times 100}{\text{Total drug}}$$

FORMULATION OF ITRACONAZOLE NIOSOMAL GEL

Niosomal suspension (10ml) containing itraconazole equivalent to 2% w/w was incorporated into the gel base composed of Carbopol 940 (1.5%), Glycerol (10%), Triethanolamine (q.s.) and distilled water up to 15ml.[9]

EVALUATION OF ITRACONAZOLE NIOSOMAL GEL[11]

Physical Appearance

Clarity, color, homogeneity and the presence of foreign particles in the niosomal gel was determined.

pH

pH of the niosomal gel was determined using digital pH meter.

Viscosity

Brookfield viscometer was used to determine the viscosity of the niosomal gel.

Drug content uniformity[12]

Drug content of the niosomal gel was calculated by dissolving 10mg of the drug in 100 ml of volumetric flask and suitable volume with 50% n-propanol was formed for lysis of the niosomes. The volume was made up to 100ml using methanol. The solution was then filtered and absorbance was measured under UV spectrophotometer at 262 nm.

Entrapment Efficiency[13]

The free drug concentration in the supernatant was determined at 262 nm using UV- Visible Spectrophotometer 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 so as to separate the niosomes from the unentrapped drug. The % drug entrapped was calculated from the formula:

$$\% \text{ drug entrapment} = \frac{(\text{Total drug} - \text{Drug in supernatant liquid}) \times 100}{\text{Total drug}}$$

In-vitro drug diffusion studies[14]:

In-vitro diffusion studies of the niosomal gel were carried out using dialysis membrane. The drug release from the niosomal gel was determined from the collected samples. The analysis of the collected samples was done under UV spectrophotometer at 262 nm.[12]

Stability studies[15-16]

Stability studies of the different formulations were carried out under different temperature conditions so as to check the effect on: physical appearance, entrapment efficiency and drug content. The niosomal formulations were stored at 2- 8°C and at room temperature (30±2°C) in air tight containers for 30 days and 2 ml samples were withdrawn every 15 days and at the end of 45 days. The analysis of the samples was then done spectrophotometrically at 262 nm after lyses of the niosomes and further preparing their suitable dilutions.

RESULT AND DISSCUSION

Drug content and Entrapment Efficiency

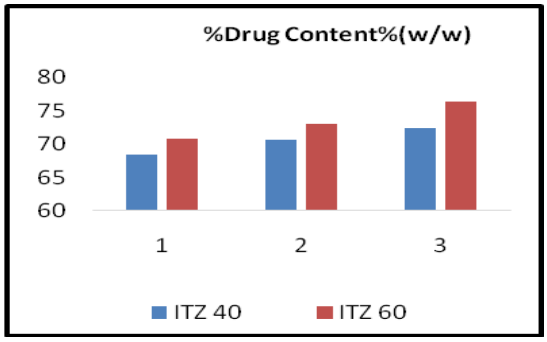

Figure.1: Drug content of ITZ 40 and ITZ 60

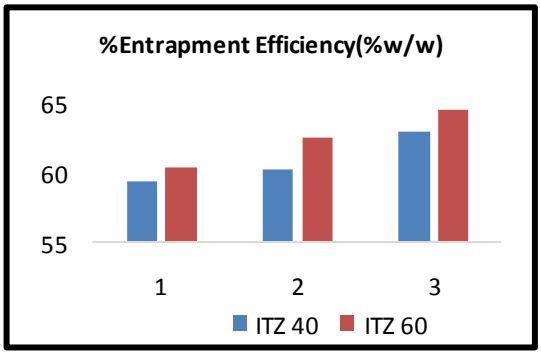

Figure 2: Entrapment efficiency of ITZ 40 and ITZ 60

The drug content was found to be 72.31% and 76.37%. The entrapment efficiency was found to be 62.9% and 64.6% for the formulations ITZ 40-3 and ITZ 60-3. The highest entrapment efficiency was observed with Span 60formulation followed by Span 40 niosomes in the similar range. . This may be due to the difference in phase transition temperature. The order of non-ionic surfactants that resulted in better entrapment efficiency is as follows: **Span 60 > Span 40**

PREPARATION AND PHYSICAL EVALUATION OF GEL CONTAINING NIOSOMES

Itraconazole niosomal gel formulations with the optimized ratio of the surfactants were prepared with Carbopol 940 as gelling agent which is due to its hydrophilic nature and bio adhesive properties, which may result in an increased residence time of drug at the site of absorption by interacting with the mucosa. The formed gel formulations were evaluated for appearance, pH, viscosity, drug content, and *in-vitro* drug release study.

Physical appearance and melting point of drug:

The formed gel is off-white in color. It is little bit sticky in nature. The melting point is observed to be 170^0C. The value is same according to the literature value.

Table: 3 Melting point of Itraconazole

Reported Value	Experimental Value			Mean Value
170^0C	169^0C	170^0C	170^0C	170^0C

pH:

The pH of the gel formulations were found to be in the range of 6.7 to 6.9.

Table: 4 pH of the different Niosomal gel formulations.

S.No	Formulation code	PH
1.	ITZ 40-3	6.54
2.	ITZ 60-3	6.25

Viscosity:

The viscosity of the gel formulations were mentioned in the table 5;

Table 5: Viscosity of the gel formulations

S.No	Formulation code	Viscosity (cps)
1.	ITZ 40-3	8569
2.	ITZ 60-3	8173

Drug content-

The percentage drug content of the formulations were mentioned in the table as follows 6;

Table: 6. % Drug content of the different gel formulations.

S.No	Formulation code	% Drug content (%w/w)
1.	ITZ 40-3	52.81
2.	ITZ 60-3	56.12

Entrapment Efficiency: The entrapment efficiency of the gel is shown in Table 7;

Table 7: Entrapment Efficiency of the different gel formulations

S.No	Formulation code	% Entrapment (%w/w)
1.	ITZ 40-3	42.20
2.	ITZ 60-3	45.20

The highest entrapment efficiency was observed with ITZ 60-3 formulation. This may be due to the higher phase transition temperature of Span 60.

Spreadibility

The Spreadibility values of all formulated gel formulations ranges from 1.50 to 5.77 g/sec. The result showed that as the conc. of surfactant increases the Spreadibility of gel formulations also increases.

Table 8: Spreadibility of the different gel formulations.

S No.	Formulation code	Spreadibility
1	ITZ 40-3	1.94
2	ITZ 60-3	2.54

In- vitro release studies of gel

Table 9: In vitro drug release study of formulation batches of F1 to F3

Sr. No.	Time (hr.)	% drug release		
		Pain gel	Niosomal gel (Span40)	Niosomal gel (Span60)
1.	0	0	0	0
2.	2	6.07±0.85	8.18±0.62	10.64±0.60
3.	4	8.30±1.60	10.92±2.10	19.42±2.60
4.	6	12.23±1.09	15.24±1.40	22.66±1.81
5.	8	18.36±0.62	22.17±0.45	27.22±2.17
6.	10	21.27±1.95	28.26±1.04	35.26±1.04
7.	12	29.28±1.02	34.17±0.47	41.66±1.58

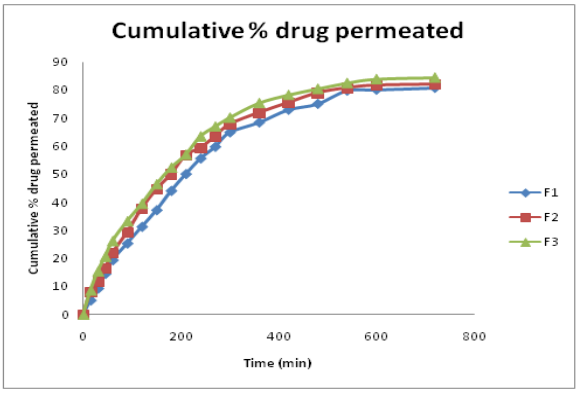

Figure 3: *In-vitro* permeation study of formulation batches of F1 to F3

Stability studies of Itraconazole niosomal gel the prepared Itraconazole niosomal gel formulations (ITZ 60-3) were tested for stability on storing them in glass vials at 4-8°C, 25°C and 40 for 45°C days. On 7th, 14th and 30 days, they were evaluated for particle size, drug content and entrapment (%). Formulations were stored at 4-8°C, 25°C and 40°C for 45 days. After 1 month of storage, the stability testing data indicated that formulations stored at 4-8°C, 25^{0}C and 40°C showed little difference in size so we can say that our formulation is stable on all temperatures.

Table: 10. Stability study of Itraconazole niosomal gel formulation (ITZ 40-3) at Different temperature.

Time of storage in days	Temperature of storage					
	Drug Content (%) 4°C - 8°C (Refrigeration tem.)	Entrapment Efficiency (%) 4°C - 8°C (Refrigeration tem.)	Drug Content (%) 25°C ±2°C (Room tem.)	Entrapment Efficiency (%) 25°C ±2°C (Room tem.)	Drug Content (%) 40°C ±2°C (Refrigeration tem.)	Entrapment Efficiency (%) 40°C±2°C (Refrigeration tem.)
0	52.81	42.10	52.81	42.10	52.81	42.10
15	51.90	43.5	51.90	42.01	52.90	41.7
30	51.71	42.2	51.16	41.5	51.83	40.9
45	50.32	41.0	50.76	41.4	51.24	40.4
60	49.89	40.2	49.86	40.4	51.02	40.2

Table 11: Stability study of itraconazole niosomal gel formulation ITZ 60-3 at Different temperature.

Time of storage in days	Temperature of storage					
	Drug Content (%) 4°C - 8°C (Refrigeration tem.)	Entrapment Efficiency (%) 4°C - 8°C (Refrigeration tem.)	Drug Content (%) 25°C ±2°C (Room temperature)	Entrapment Efficiency (%) 25°C ±2°C (Room tem.)	Drug Content (%) 40°C ±2°C (Refrigeration tem.)	Entrapment Efficiency (%) 40°C ±2°C (Refrigeration tem.)
0	56.12	45.2	56.12	45.2	56.12	45.2
15	55.31	44.7	55.19	45.1	55.34	44.4
30	54.30	44.2	55.17	44.9	55.21	43.3
45	54.29	44.19	55.06	44.7	54.32	42.2
60	54.28	44.01	55.01	44.4	54.21	41.5

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. Reverse phase evaporation method 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 40 and Span 60) and keeping the cholesterol concentration fixed. The

order of entrapment efficiency of the formulations are as follows **SPAN 60 > SPAN 40.**The formulated niosomes (ITZ 40-1, ITZ 40-2, ITZ 40-3 and ITZ 60-1, ITZ 60-2, ITZ 60-3) were characterized for drug content and entrapment efficiency. Out of six formulations the two formulation (ITZ 40-3 and ITZ 60-3) were selected for further evaluation study. The optimized Niosomal dispersion were further formulated as topical gel delivery system. The formulated niosomal gel was characterized for the physical appearance, pH and rheological behaviour. The order of *in- vitro* release of the niosomal gel formulations are as follow **SPAN 40 < SPAN 60.** From the stability studies, it was found that by using optimum storage condition, niosomal gel formulations was stable on all temperatures. There was only slight difference in drug content and drug entrapment. It is concluded that the Reverse phase evaporation method is a useful method for the successful incorporation of poorly water soluble drug Itraconazole into niosomes with high entrapment efficiency. 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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