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Preparation and evaluation of itraconazole liposome using ether injection solvent evaporation method

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Abstract

Itraconazole is an antifungal drug, is a frequently and widely used drug to treat fungally infected skin. The presently available ITZ formulations for topical use, with their traditional design formulation pattern however, maintaining their place in the amount of dermatologists but now evoked the thought of their improvement in the light of the latest developments. Liposomes with their potential promise to improve the transportation of ITZ to make it better localized and acted upon the micro-organisms have been valued in the present investigation. A facilitated bio-friendly vehicular effect, active hydrating conditions and a provision for a solubilized status of drugs are some obvious advantages of these vesicular systems. The work on the development of liposomes of ITZ was taken-off by focusing on the studies to gain the knowledge on drug-characters (i.e., drug profile) and estimation techniques. The techniques to determine the drug concentration during various studies were studied out (e.g., drug entrapment, *in-vitro* characterization and evaluation). A diffusion medium for drug release was studied for ITZ. From the results obtained it was concluded that out of four formulations prepared, F3 was optimized formulation.

Key- words: Itraconazole, Fungal infection, liposomes

Introduction

A liposome is a tiny bubble (vesicle), made out of the same material as a cell membrane. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases. Liposomes were first described by British haematologist Dr Alec D Bangham FRS in 1961 (published 1964), at the Babraham Institute, in Cambridge. They were discovered when Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids. The resemblance to the plasmalemma was obvious, and the microscope pictures served as the first real evidence for the cell membrane being a bilayer lipid structure.[1-2]

Itraconazole [3-4]

Itraconazole is an antifungal medication used to treat a number of fungal infections. This includes aspergillosis, blastomycosis, coccidioidomycosis, histoplasmosis, and paracoccidioidomycosis. It may be given by mouth or intravenously. Itraconazole is a triazole antifungal agent that inhibits cytochrome P-450-dependent enzymes required for ergosterol synthesis.

Itraconazole is a synthetic triazole agent with antimycotic properties. Formulated for both topical and systemic use, itraconazole preferentially inhibits fungal cytochrome P450 enzymes, resulting in a decrease in fungal ergosterol synthesis. Because of its low toxicity profile, this agent can be used for long-term maintenance treatment of chronic fungal infections.

Chemical formula: $C_{35}H_{38}Cl_2N_8O_4$

IUPAC: 2-butan-2-yl-4-[4-[4-[4-[[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-1,2,4-triazol-3-one

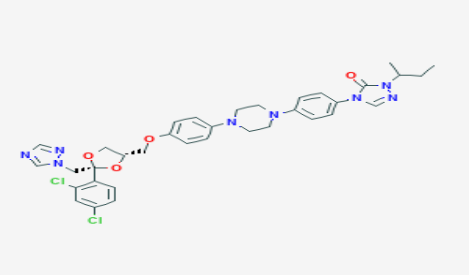

Fig. 1: Structure of Itraconazole

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Fungal infections are of immense global significance as around 30% of world's population experiences fungal infections. The tropical countries such as India are more prone to the fungal infection and around 2million cases are reported annually. Existing treatments for fungal infection include a limited number of clinically effective antifungal agents. However, the clinical utility of the most of the antifungal agents is hampered due to problems such as poor oral bioavailability and the emergence of drug-resistant against fungal strains. On the other hand, despite numerous efforts and investigations, there are no effective and promising antifungal drugs on the horizon till date. This scenario has enforced the smart and effective utilization of the current antifungal agents with the help of novel drug delivery systems i.e., liposomes. Therefore, the present work was undertaken to formulate the liposomes of ITZ.

Material and Methods

Material

Itraconazole was a gift sample from Dr. Reddy's lab Pvt. Ltd, Hyderabad, India. Soya lecithin, Cholesterol was obtained from Sigma Aldrich and Cholesterol from SD Fine. All other materials used in this study were of analytical grade.

Formulation of Itraconazole Liposomes (ether injection solvent evaporation method)

Liposomes were prepared by solvent injection method using different formulations as shown in Table 1. In a beaker weighed quantity of Lecithin and Cholesterol were taken and dissolved in 10mL of di-ethyl ether (Lipid phase). In another beaker specified amount of drug is dissolved in 10mL of methanol and to this 10 mL of buffer is added (Aqueous phase). The beaker with aqueous phase is kept on for stirring at 200 rpm on thermostatically controlled magnetic stirrer (Remi Magnetic Stirrer, Model: LBMS-5886) at a temperature of 45 ºC. The beaker with lipid phase was also kept aside on the magnetic stirrer to attain 45°C temperature. To this aqueous phase at 45°C lipid phase which is also at 45°C was added by injection at one jet. The mixture was continued for stirring for 1 hour to obtain uniform vesicular dispersion. Finally the liposome dispersion was stored in airtight container at 2-8°C.[5-7]

Preparation of Liposomal hydrogel

Itraconazole liposomal hydrogel were prepared by incorporating formulated liposomes into 2% carbopol 934. Weighed quantity of the carbopol 934 was dissolved in 15 mL of distilled water and stirred thoroughly to get homogeneous slurry. The itraconazole liposomal formulations were incorporated into the prepared carbopol gels and missed thoroughly and the pH was adjusted to neutral with triethanolamine.[8-9]

Evaluation of Liposomes[10-14]

Physico-chemical Characteristics

The formulated liposomal gel was evaluated for color, odor, pH immediately after preparation and on 15th day.

Optical microscopy

The prepared Itraconazole liposomes were viewed under for observing the vesicle formation and discreteness of dispersed vesicles. A slide was prepared by placing a drop of liposome gel on a glass slide and cover slip was placed over it and this slide was viewed under optical microscope at 40X magnification. Photographs were taken to repared slides using digital camera.

Particle size determination

The particle size determination is done by using Horibu nano particle analyser and the method used here was Dynamic Light Scattering method. Dynamic Light Scattering (DLS also known as Photon Correlation Spectroscopy or Quasi-Elastic Light Scattering) is one of the most popular light scattering techniques because it allows particle sizing down to 1 nm diameter. The basic principle is simple: The sample is illuminated by a laser beam and the fluctuations of the scattered light are detected at a known scattering angle θ by a fast photon detector. Particle size determination of liposomes was done by using Optical microscope. Determination of particle size as mean diameter is based on direct observation under microscope. The procedure includes 2 steps:

Calibration of eye-piece micrometer

The eye piece micrometer contains 100 divisions. The determination of actual length of each division is known as calibration. This is done in comparison with the standard stage micrometer. The number of divisions of eye piece micrometer (x) matching equally with the number of divisions in stage micrometer (y) was noted. Each division of stage micrometer is equal to 0.01 mm or 10 micrometer. Now one division of eye piece micrometer is equal to number of divisions of stage micrometer (y) divided by number of divisions of eye piece micrometer.

Measurement of globule size

A droplet of liposome formulation was mounted on glass slide and placed on mechanical stage of microscope then the globule diameter was measured and recorded for 100 globules and average particle size was determined.

Drug entrapment efficiency

The drug entrapment efficiency was calculated using the total drug content of liposome gel and

unentrapped drug content of the liposomal gel. The total dug content is determined estimating total drug entrapped and unentrapped. 5 ml of liposome gel was taken in a volumetric flask. The gel was subjected to sonication in bath sonicator (M/s. Remi) for 30 minutes. Then the mixture was filtered and estimated after suitable dilution at 280 nm wavelengths by using UV Visible Spectrophotometer (Shimadzu, UV1800). For the free unentrapped drug, 5 ml of the liposome gel subjected to centrifugation at 18000 rpm using Remi centrifuge for 40 min at 50 C. The supernatant clear solution was collected separately and the free drug present in the supernatant was estimated after suitable dilution at 255 nm wavelength by using UV Visible Spectrophotometer.
The entrapment efficiency of all the formulation was calculated by using following formula.

% Entrapment Efficiency = Amount of drug entrapped/total amount of drugX100

***In vitro* diffusion studies**

In vitro diffusion studies were carried by using Franz diffusion cell apparatus. The capacity of the receptor compartment was 20 ml and the area of the donor compartment exposed to receptor compartment was 1.41cm2. Dialysis membrane-50 with molecular weight cut off 12000 to 14000 Da from Hi-Media Laboratories Pvt. Ltd having flat width of 24.26 mm and diameter of 14.3 mm with approximate capacity of 1.61 mL/cm was used for the study. The membrane was soaked overnight in phosphate buffer pH 7.4. 10 ml of prepared liposomal gel which contains 10 mg of drug was taken and placed in the donor cell. Dialysis membrane was placed in between donor cell and receptor cell. 20 ml of phosphate buffer (pH 7.4) was taken in receptor cell to touch the bottom surface of dialysis membrane. The temperature of the receptor phase was maintained at 37 ± 0.5° C and the receptor compartment was stirred with magnetic stirrer to maintain homogeneous condition. The aliquots of 3 ml were withdrawn at different time intervals. Fresh medium was used to replace with equal volume of the sample withdrawn. The samples were analyzed at 255 nm in a UV-Visible spectrophotometer and amount of drug released at different time intervals was calculated.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy was used to characterize the surface morphology of the prepared vesicles. One drop of liposomal gel was mounted on a clear glass stub, air-dried, coated with Polaron E 5100 sputter coater (Polaron, Watford, United Kingdom), and visualized under a scanning electron microscope (Leo-435 VP; Leo, Cambridge, United Kingdom.

Table 1: Composition of liposome formulations of itraconazole by ether injection method

Formulation Code	Composition		
	Drug (mg)	Lecithin (mg)	Cholesterol (mg)
F1	50	100	50
F2	100	100	50
F3	150	50	100
F4	200	50	100

Results and Discussion

Physico-chemical Characteristics

The liposomal formulation was evaluated for the physic-chemical properties. All the prepared formulation was found to be colorless and odorless. The liposomal hydrogel were also found to be odorless, translucent and with neutral pH. The characteristics of the formulation prepared and after 15th day did not showed any difference, it indicates the physical stability of the prepared formulation. The physic-chemical behavior of itraconazole liposomal gel suggests that it has a good potential for topical drug delivery for the treatment of fungal diseases.

Optical microscopy

The vesicles were observed under optical microscope was found to be discrete and spherical in shape. The images (shown in Fig 5.1) clearly indicated the discrete structures of liposomes vesicles for the formulation F1 to F4.

Particle size determination

The mean particle size of the itraconazole liposomal formulation was found to be 92.87$\pm$2.5 to 106.72$\pm$3.9 nm. The formulation F2 has low particle size and it was observed that as the concentration of cholesterol increases the particle size also increase.

Drug entrapment efficiency (EE)

The amount of drug entrapped into the liposome gel was determined. The entrapment efficiency was found to be in the range of 84.32 to 98.29%. From the results it was concluded that a good amount of drug was entrapped in the liposome formulation prepared.

***In vitro* diffusion studies**

The Itraconazole Liposomes gels were subjected to *in vitro* diffusion studies and the results indicated that the formulation F-3 showed highest drug release of 82.31% w/w.

Scanning Electron Microscopy (SEM)

The scanning electron microscopy describes the surface morphology of Liposomes and SEM of optimized formulation F3 is hown in fig. 5.2. It was inferred from the SEM images that the liposomes were spherical and smooth vesicular structures.

Conclusion

From the results obtained it was concluded that out of four formulations prepared, F3 was optimized formulation. The benefits and outcome of the present work is:

- The development of novel drug delivery systems such as liposomes for the drugs, ITZ could be materialized successfully. And they were characterized for their vesicle-specific properties.
- The developed systems could fulfill the delivery-objectives to a satisfactory extent.

References

1. Vyas S.P., Khar K.R. Targeted and Controlled drug delivery. CBS Publisher and distributors, New Delhi. 2002; 1; 181-187.
2. Riaz.M. Liposomes preparation method . Pakisthan Journal of Pharmaceutical Sciences. 1996; 19(1); 65-77.
3. https://medlineplus.gov › Drugs, Herbs and Supplements
4. https://www.webmd.com/drugs/2/drug-128-2179/itraconazole-oral/itraconazole-oral/details
5. A Laouini, C Jaafar-Maalej, I Limayem-Blouza, S Sfar; C Charcosset, H Fessi, State of the Art. *Journal of Colloid Science and Biotechnology*, 2012, 1, 147–168.
6. Srinivas Lankalapalli, Venkata Satya Vinai Kumar Tenneti, Sunil Kumar Nimmali, *Indian Journal of Pharmaceutical Education and Research*, 2015, 49 (3), 208-215.
7. Anamarija Ćurić, Regina Reul,Jan MöschwITRer, Gert Fricker, *International journal of pharmaceutics*, 2013, 448 (1), 189-197.
8. Sunitha Sampathi, Tirupathi Ajimera, Vijaya kuchana, *Journal and pharmaceutical and scientific innovation*, 2014, 3(1), 25-29.
9. Tang J, Wei H, Liu H, Ji H, Dong D, Zhu D, Wu L. *Drug Deliv*, 2010, 17(4), 223-230.
10. L. Srinivas, VS Vinai kumar tenneti, B.N Malleswara Rao, B. Bhanu Teja, *American journal of Pharmatech research*, 2014, 4(2), 827-847.
11. Lian, T., Ho, R.J.Y. Trends and developments in liposome drug delivery systems. J Pharm Sci 2001; 90: 667-680.
12. Mahesh N., Varsha B. Pokharkar. Development of vitamin loaded topical liposomal formulation using factorial design approach: Drug deposition and stability. International Journal of Pharmaceutics 2006; 320: 37-44.
13. Gomez-Hens, Analytical methods for the control of liposomal delivery systems. Trends in Analytical Chemistry 2006; 25: 2.
14. Maitani Y, Igarashi S. Cationic liposome (DC-Chol/DOPE = 1:2) and a modified ethanol injection method to prepare liposomes increased gene expression. International Journal of Pharmaceutics 2007; 342: 33-39.

Table 2: Physicochemical characteristics of itraconazole liposomes

S/No.	Parameters	F1	F2	F3	F4
1.	Color	Colorless	Colorless	Colorless	Colorless
2.	Odor	Odorless	Odorless	Odorless	Odorless
3.	pH	7.1	6.89	7.0	7.0

Table 3: Physicochemical characteristics of itraconazole liposomal gel

S/No.	Parameters	F1	F2	F3	F4
1.	Color	Colorless	Colorless	Colorless	Colorless
2.	Odor	Translucent	Translucent	Translucent	Translucent
3.	pH	7.0	7.0	7.0	7.0

Table 3: Particle size of itraconazole liposomal gel

S/No.	Formulation Code	Particle size (nm) (Mean±SEM; n=100)
1.	F1	92.87±2.5
2.	F2	98.25±2.1
3.	F3	103.46±1.8
4.	F4	106.72±3.9

Table 4: Drug entrapment efficiency of itraconazole liposomal gel

S/No.	Formulation Code	Drug entrapment efficiency (%)
1.	F1	84.32
2.	F2	91.43
3.	F3	98.12
4.	F4	98.29

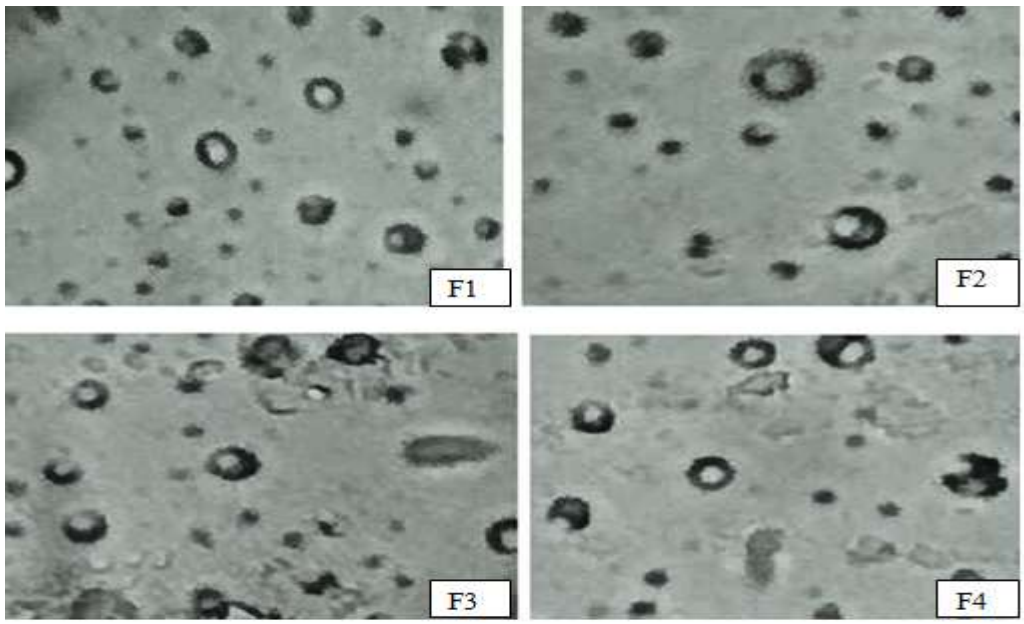

Fig. 2: Optical microscopy of formulation code F1 to F4

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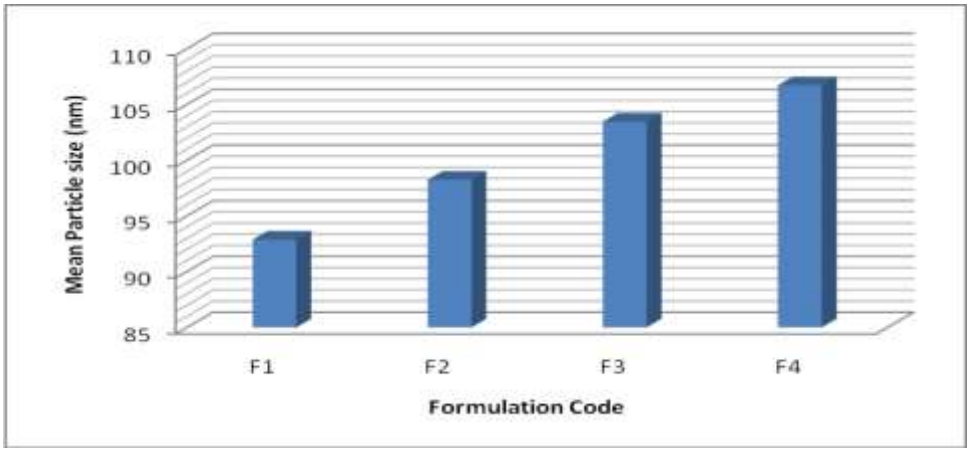
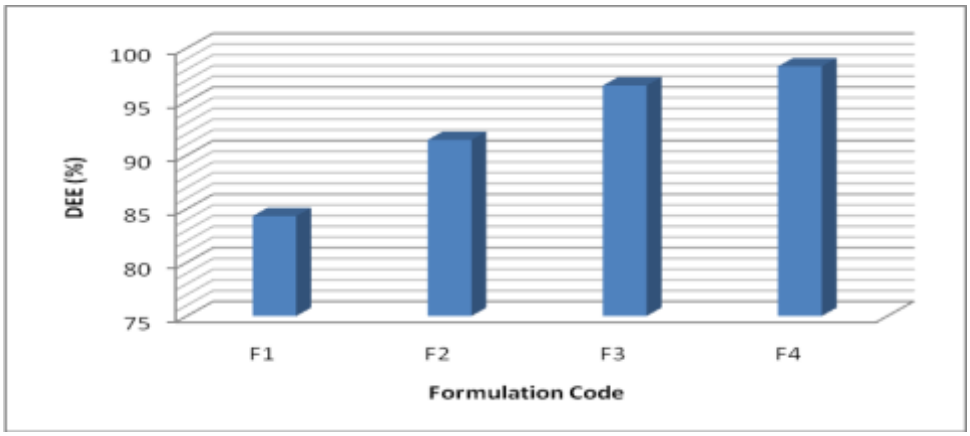


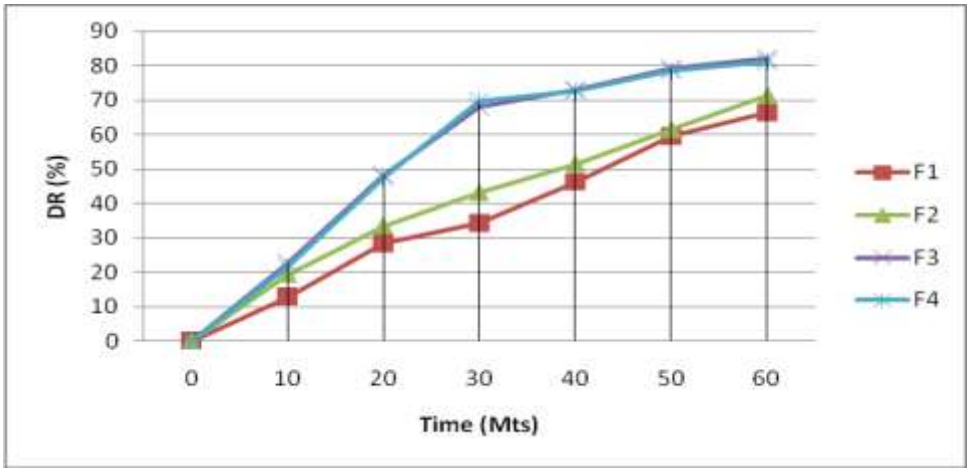
Graph 1: Mean Particle size of itraconazole liposomal gel



Graph 2: Drug entrapment efficiency of itraconazole liposomal gel

Table 5: *In vitro* diffusion studies of itraconazole liposomal gel

S/No.	Time (in mts)	Formulation Code			
		F1	F2	F3	F4
1.	0	0	0	0	0
2.	10	12.83	19.43	22.76	21.49
3.	20	28.34	33.46	48.27	47.54
4.	30	34.19	43.17	68.19	69.72
5.	40	46.17	51.43	73.14	72.46
6.	50	59.43	61.40	79.30	78.48
7.	60	66.40	71.29	82.31	81.10



Graph 3: *In vitro* drug release of itraconazole liposomal gel

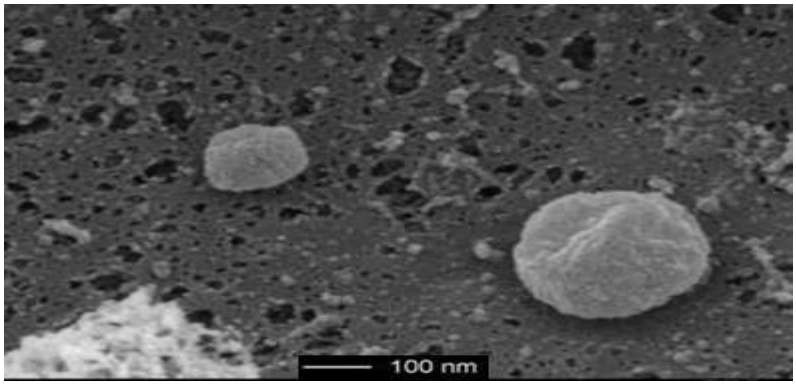


Fig. 3: SEM of optimized formulation F3

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