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Formulation and Evaluation of Fluconazole Loaded Elastic Liposomes for the Treatment of Keratomycosis

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Abstract

The present study was aimed at formulating and evaluating fluconazole loaded elastic liposomes to treat keratomycosis. The elastic liposomes were formulated using thin film hydration method, by utilising different concentrations of phospholipids and different edge activators. The elastic liposomes prepared were evaluated for different parameters such as % entrapment efficiency, in vitro drug release study, polydispersity index, size and shape of vesicles. Formulation E2 was optimized on the basis of % entrapment efficiency and in vitro drug release study. The % entrapment efficiency of the optimized formulation was estimated to be 95.2, % cumulative drug release was 91.35±1.4, polydispersity index was 0.303 ± 0.03, vesicles size was 173.6± 5.9 nm and the shape of the vesicles was spherical with smooth surface. These results verified that the fluconazole loaded elastic liposomes formulated were effective against keratomycosis.

Keywords: Elastic liposomes, Fluconazole, Keratomycosis, Ocular delivery

Introduction

Elastic liposomes are lipid bi-layered vesicles composed of phospholipids and edge activator. They are capable of delivering numerous drugs (which can be hydrophilic or hydrophobic) and can be administered by different routes such as topical, transdermal, nasal, etc. The water soluble drug is encapsulated in an hydrophillic chamber which is surrounded by double layer of phospholipids, while the lipophilic drug is encapsulated within the lipid bi-layer [1].

They are known elastic liposomes due to the elasticity of the lipid bi-layer. The edge activator provides elasticity to the lipid membrane. The edge activator reduces the transition temperature of the phospholipids thus resulting in destabilization of the phospholipids bi-layer causing increased fluidity/ elasticity of the bi-layer [2].

Keratomycosis is an ocular disease caused by fungus. In this fungus infects the cornea and can involve any part of cornea. It normally occurs due to injury and is demarcated by an ulcer which is fluffy white and appears as raised projection encircled by superficial cavity on the border. The symptoms may include pain, tearing, foreign body sensation, etc [3].

According to WHO, the major cause of blindness is keratomycosis and all over 1.5 to 2 million new cases are being announced yearly. Its soaring occurrence is accredited to tropical climate and agriculture as prime occupation and Indians are at soaring danger due to soaring exposure for work trauma [3].

Fluconazole is a triazole derivative and is an antifungal agent. It is used to treat various fungal infections such as dermatophytosis, keratomycosis, candidiasis, etc.

Fluconazole was chosen for the current study due to the following reason:

- Due to its broad spectrum activity [5].
- It inhibits the fungal cytochrome P-450 enzymes, which are found to be lower in mammalian enzymes and more in fungal species, thus improving its safety profile [5].
- It is a potential drug for appraisal as a antifungal agent for topical application in the eye due to its improved penetration in the aqueous humour as well as reduced toxicity [6].
- Being B.C.S class II drug, it has slight solubility [7].
- The side effects caused by the oral administration of drug such as hepatotoxicity, GIT disturbance, etc can be avoided by administering the drug through topical route [8].

From the literature review, it was observed that many other formulations of fluconazole have been prepared but they all were having some drawbacks. Some of the formulations are given below in table 1 along with their drawbacks.

No elastic liposomes of fluconazole have been reported yet. So fluconazole loaded elastic liposomes were prepared.

Materials and Methods

2.1. Chemicals and reagents

Fluconazole was provided kindly by Ramson Remedies, Amritsar. Soya lecithin was purchased from Hi Media, Mumbai, India. Tween 80 was purchased from Qualikems, Vadodara, India, sodium deoxycholate was purchased from Hi Media and span 80 was purchased from Central Drug House, New Delhi, India. All the other chemicals and reagents were of analytical grade and procured from Merck, Mumbai, India.

2.2. Formulation Development

2.2.1. Solubility studies

Solubility of fluconazole was determined in various solvents such as methanol, chloroform, simulated tear fluid pH 7.4, ethanol, etc which were to be used during formulation development and in vitro characterization of the formulation. For the determination of solubility of drug, the different solvents were taken in different eppendorfs and an excess amount of drug was added in them. The eppendorfs were placed in an isothermal shaker at 25° C for

72 hours. After 72 hours the samples were centrifuged for 15 min at 3000 rpm. The supernatant was separated, diluted and filtered. Using UV spectrophotometer, the concentration of drug in supernatant was determined [11].

2.2.2. Preparation of Elastic liposome

The conventional rotary evaporation method was used for the formulation of elastic liposomes. [12]. Different concentrations of phospholipids and different edge activators in varying ratios were used for the formulation of elastic liposome. For the formulation of different EL batches, accurately weighed amount of phospholipid (Soya lecithin), drug and surfactant were taken in round bottom flask and were dissolved in optimized amount of organic solvent (chloroform). The organic solvent was evaporated at 40° C and reduced pressure using rota evaporator. A thin film was obtained at bottom of round bottom flask after evaporation of organic solvent. The film obtained was hydrated using distilled water (10 ml). The suspension obtained was stored in vial.

2.3. Evaluation of Elastic liposomes

The formulated elastic liposomes were evaluated for:

2.3.1. ***% Entrapment efficiency***

The prepared suspension of elastic liposomes was centrifuged at 15,000 rpm at 4º C using cooling centrifuge for 40 min. The supernatant obtained was separated and after suitable dilution of supernatant with methanol, the free drug in the supernatant was assayed using UV spectrophotometer at 261 nm. The entrapment efficiency was calculated using the formula given below:

$$\% \text{ Entrapment Efficiency} = \frac{(Wt - Wf) * 100}{Wt}$$

Where, % EE, Wt and Wf are the drug entrapment efficiency, total amount of fluconazole in elastic liposome suspension and free fluconazole in the supernatant respectively [13].

2.3.2 In vitro drug release study

The study was performed using a Franz diffusion cell using treated dialysis membrane. The receptor compartment was filled with simulated tear fluid pH 7.4. The membrane was placed between the donor compartment and the receptor compartment. The assembly was maintained at 37 ± 1º C and was continuously stirred using magnetic bead [14]. One ml of the formulation was loaded. At predetermined time intervals the aliquots were withdrawn and the amount withdrawn was replaced by fresh medium. The amount of drug was calculated using UV spectrophometer at 261nm [15].

2.3.3. Polydispersity index and vesicle size

The polydispersity index and size of vesicle of the optimized formulation was evaluated using Zetasizer. The sample was maintained at 25º C ± 2º C and the beam was adjusted at a scattering angle of 90 º [16].

2.3.4. Vesicle shape

The shape of the vesicles of the optimized formulation was observed under olympus microscope.

3. Results and discussion

3.1. Formulation development

3.1.1. Solubility studies

Solubility of fluconazole in different solvents was determined as per the procedure and was found to be 68.5± 1.2 in chloroform, 64.8±0.85 in acetone, 62± 0.8 in ethanol, 165± 0.12 in methanol and 5± 0.45 in simulated tear fluid.

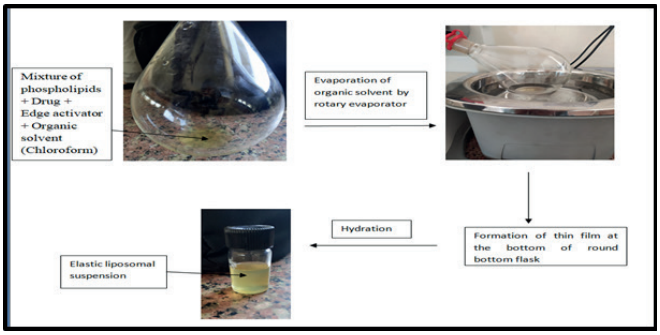

Figure 1: The steps involved in the preparation of drug loaded elastic liposomes

Solubility in chloroform, acetone, ethanol was determined for formulation development. The solubility was found to be highest in chloroform (68.5± 1.2), so chloroform was used in formulation development.

Solubility in methanol and simulated tear fluid pH 7.4 was determined for in vitro studies. Solubility in methanol was 165± 0.12 and in STF was 5± 0.45

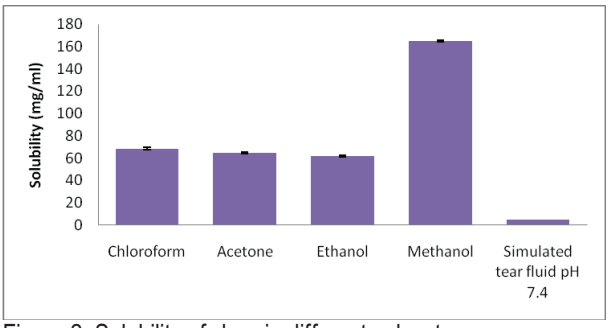

Figure 2: Solubility of drug in different solvents

3.1.2. Preparation of elastic liposomes

The elastic liposomes were prepared by thin film hydration method (Figure 1) by differing concentration of phospholipids (3-5%), drug (50 mg) and using three edge activators (tween 80, span 80 and sodium deoxycholate). Table 2 shows the composition of the elastic liposomes formulated.

3.2. Evaluation of prepared formulation

3.2.1. % Entrapment efficiency (% EE)

The % entrapment efficiency was determined as per the procedure and % EE of the prepared formulations was found to be 90.8 of E1, 95.2 of E2, 89.2 of E3, 77.4 of E4, 74.9 of E5, 82.2 of E6, 74.2 of E7, 83.8 of E8 and 41.2 of E9.

The maximum % EE was found to be of E2 (95.2), the formulation composed of 4% phospholipids and sodium deoxycholate.

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Table 1: The drug delivery systems of fluconazole with their drawbacks

Drug Delivery System Formulated	Drawbacks	Reference
Creams and ointments	Poor patient compliance due to stickiness and blurred vision	[8]
Aqueous eye drops	Insufficient residence time due to which only 5% drug dose get access to intraocular tissues.	[9]
Solid unit dosage form, I.V. Injection, Powder for oral suspension	Hepatotoxicity , stomach pain, Fast/ irregular heartbeat, skin rashes, Tightness in chest, chills	[10]

Table 2: Composition of drug loaded elastic liposomes

Formulation code	Drug (mg)	Phospholipids (%)	Sodium deoxycholate (mg)	Tween 80 (mg)	Span 80 (mg)
E1	50	3	✓	-	-
E2	50	4	✓	-	-
E3	50	5	✓	-	-
E4	50	3	-	✓	-
E5	50	4	-	✓	-
E6	50	5	-	✓	-
E7	50	3	-	-	✓
E8	50	4	-	-	✓
E9	50	5	-	-	✓

Table 3: % Entrapment Efficiency of the formulations prepared

S. No.	Formulation Code	% Entrapment efficiency (%EE)
1	E1	90.8
2	E2	95.2
3	E3	89.2
4	E4	77.4
5	E5	74.9
6	E6	82.2
7	E7	74.2
8	E8	83.8
9	E9	41.2

Table 3 shows the results of the % EE of various formulations prepared.

3.2.2. In vitro Drug release study

The study was performed as per the procedure mentioned above. The in vitro release profiles of the different formulations formulated were compared with the in vitro release profile of solution of drug. The % cumulative drug release of the formulation E1 was 69.65±1.75, E2 was 91.35±1.4, E3 was 85.05±1.45, E4 was 74.55±1.42, E5 was 86.1±1.54, E6 was 79.45±1.4, E7 was 71.4±1.39, E8 was 75.6±1.19, E9 was 63.7±1.27 and of drug solution was 98.7±1.25. The % cumulative drug release of formulation E2 was found to be highest among other formulations and could be correlated with the % EE. The % cumulative drug release of optimized formulation E2 was found to be less than % cumulative drug release of drug solution.A higher release of drug from drug solution was due to the presence of drug in its free form resulting in its easy diffusion. The prepared elastic liposome suspension showed a sustained and continuous release of drug over a period of 6 hours. The slow release of drug from elastic liposomes can be attributed to the chemical characteristics of drug, which allowed a certain affinity of drug with vesicular structure or the chemical components (phospholipids and surfactant) of elastic liposomes.

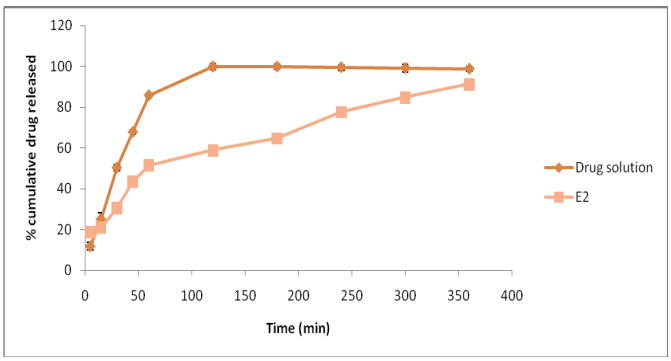

Figure 3: In vitro release profile of optimised formulation E2 and Drug solution

Figure 3 shows the comparison of in vitro release profile of optimized formulation and drug solution .

3.2.3. Polydispersity index and vesicle size

The optimized formulation (E2) exhibited a polydispersity index of 0.303 ± 0.03. The polydispersity index was found to be within the range (< 0.5), which suggested the uniformity and homogeneity of the formulation. The vesicle size of the optimized formulation (E2) was to be 173.6± 5.9 nm.

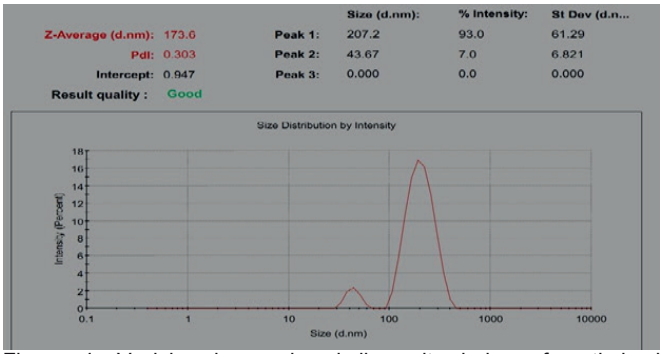

Figure 4: Vesicle size and polydispersity index of optimized formulation E2

Figure 4 shows the image of graph of polydispersity index and vesicle size of optimized formulation (E2).

3.2.4. Vesicle shape

The vesicle shape was found to be spherical and was having smooth surface. The lipid bi-layer was clearly visible under Olympus microscope at resolution power 10x.

Figure 5: Image of vesicle under Olympus microscope

Figure 5 shows the image of vesicle observed under Olympus microscope at resolution power 10x.

4. Conclusion

The fluconazole loaded elastic liposomes were successfully formulated. The optimized formulation E2 showed highest entrapment efficiency and % cumulative drug release with vesicles of satisfactory size. The polydispersity index was found to be within the range and the vesicles were spherical in shape with smooth surface.

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