Research Article

Formulation and Evaluation of Ketoconazole Proliposomal Gels

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Received: 12-06-2022; **Revised:** 20-10-2022; **Accepted:** 26-10-2022; **Published on:** 15-11-2022.

ABSTRACT

The goal of this research was to produce a proliposomal formulation of ketoconazole, an antifungal medication, to treat dandruff. Thin-film hydration was used to create proliposomes with various mannitol, phospholipid, and cholesterol contents (vacuum rotatory evaporator). Based on medication content, entrapment efficiency, and average size, prepared proliposomal formulations were optimised. As part of the carbopol gel formulation, the newly created proliposomal formulation was evaluated for its rheological characteristics and in vitro drug release tests. The concentrations of phospholipids and cholesterol in formulations were shown to be critical to their optimal entrapment efficiency. Stability of the carbopol-coated proliposome formulation for topical medication administration was determined by rheological experiments. Proliposomal gel formulations, as compared to pure medicines, show greater skin penetration and sustained release in vitro.

Keywords: Proliposomes, Ketoconazole, film hydration, anti-fungal, Rheological studies.

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DOI:
10.47583/ijpsrr.2022.v77i01.034

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v77i01.034

INTRODUCTION

The creation of a new drug delivery system (NDDS) has focused significant attention over the previous few decades. Ideally, the NDDS should fulfil two requirements. First, it should supply the medicament throughout the therapy at a pace that is guided by the body's requirements. Secondly, the active entity should be channeled to the action site. None of these can be met by conservative dosage forms, including prolonged-release dosage forms either. At present, no drug delivery scheme is ideal, but genuine efforts have been made to accomplish this through numerous new methods in the delivery of drugs [1-3].

The novel drug delivery system aims to provide some control of the release of drugs in the body, whether temporal or spatial in nature or both. In recent times, vesicles have become the preferred method of transporting medicines. In diagnostic methods, membrane biology, immunology, and genetic engineering, lipid vesicles have been discovered to be of importance. Vesicles can play an important role in the modelling of biological membranes as well as in transporting and targeting active medicaments. It is likely that putting a drug in vesicular structures will make the drug last longer in the body's bloodstream and may reduce its toxicity if selective uptake is possible.[4] The phagocytic uptake of the systemic transmission of the drug-loaded vesicle delivery system offers an effective method for delivering the drug straight to the infection site, resulting in reduced drug toxicity without adverse effects [5].

Because ketoconazole is classified as Class II in the Biopharmaceutical Classification System, which includes a class of low solubility and high permeability, pharmaceutical researchers are concerned about its increased solubility. Due to the obvious low solubility and side effects, oral absorption of ketoconazole is not optimal. To overcome the insufficiency of this conventional system, a new drug delivery system is required. Today, there are so many topical ketoconazole preparations available on the market, but they have some demerits. Viz cream has side effects such as rash, irritation, pain, itching, and redness. Therefore, to overcome this problem requires a new drug delivery system such as proliposomal. Proliposomal has been widely used as a vehicle in topical medicine and it is an alternative to insoluble, topical, or oral drugs [6-7].

MATERIALS AND METHODS

Divis Laboratories, Hyderabad, gathered Ketoconazole as a donation sample. Phosphatidyl Choline, Mannitol, Cholesterol, Mannitol, Carbopol 934 was purchased from SD Fine Chemicals, Mumbai. All other chemicals and reagents used were analytical grade.

Preparation of Ketoconazole embedded

Proliposomes were generated via film deposition on a carrier in a vacuum rotary evaporator. By varying the amounts of phosphatidylcholine and cholesterol, several

formulations of ketoconazole proliposomes were generated. Mannitol was put in a 100 ml round-bottom flask that was kept at 60-700°C and spun at 80-100 rpm under vacuum for 30 minutes. After complete drying, the water bath temperature was decreased to 20–300 °C. Ketoconazole, Phosphatidyl Choline, and Cholesterol were dissolved in a mixture of organic solvents (chloroform: methanol, 6:4, v/v), and 5 ml of the organic solution was gently pumped into the flask via the solvent input tube. After the drying process was completed, a second aliquot of 5 mL was added. The vacuum was then released, and the proliposomes were put in a desiccator overnight before sieving through a 100-mesh sieve. The powder was transferred to a glass container and stored in the freezer.[8–10].

Table 1: Formulation Design

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ketoconazole (mg)	10	10	10	10	10	10	10	10	10
Phosphatidyl Choline (mg)	100	100	150	150	50	150	100	50	50
Cholesterol	150	100	50	100	100	150	50	50	150
Mannitol (g)	1	1	1	1	1	1	1	1	1
Chloroform(ml)	6	6	6	6	6	6	6	6	6
Methanol(ml)	6	6	6	6	6	6	6	6	6

Preparation of Carbopol Gel Base

Carbopol 934 was weighed and then dissolved in distilled water. The mixture was then neutralised with 1 percent triethanolamine, which was applied drop by drop. After mixing until a transparent gel was created, it was left to grow for 24 hours. Similarly, 2 and 3 percent carbopol gels have been created. [11].

Preparation of Proliposomal Gels

Using a mortar and pestle, proliposomes containing ketoconazole (taken from the unencapsulated drug) were mixed into a 2 percent carbopol gel at a concentration of 1%. All of the top-performing formulae were placed in various carbopol gels (1 percent and 3 percent) [12–14].

Characterization of Proliposomes Vesicle Size and Count

Using an optical microscope, the size distribution and average size of proliposomes were determined. On a glass slide without a coverslip, a drop of distilled water was added to proliposome granules, which were then viewed using a 100 X optical microscope. On the slide, the size of liposomal vesicles was assessed in several regions. To evaluate liposome vesicles' average size and dispersion, the collected data was used [15].

Surface Morphology

SEM was used to analyse the surface morphology of proliposomes and mannitol particles after coating them with gold. The surface morphology of gold-coated proliposomes and plain mannitol particles was studied and photographed [16-17].

Drug Content

Using a UV-visible spectrophotometer, the ketoconazole concentration in proliposomes was determined. 100 mg of proliposomes were dissolved in 10 ml of methanol by vigorously shaking the liquid for 5 minutes. One millilitre of the resultant solution was used to dilute ten millilitres of methanol. Then, aliquots were taken, and the absorbance at 323 nm was measured with a UV-visible spectrophotometer (Lab India3200)[18].

Entrapment Efficiency

Centrifugation was employed to remove the unentrapped medicine from the liposomal solution. After hydration with distilled water, the entrapment efficacy of proliposomes was evaluated. Subsequently, 10 ml of phosphate buffer (pH 7.4) was added to proliposome granules, which were then ultrasonically treated for 10 minutes (Citizen, India). To remove the unencapsulated drug, the liposomal solution was spun for 30 minutes at 15000 rpm using a cooling centrifuge (REMI TR-01) 19. The absorbance at 315 nm was measured using a UV-visible spectrophotometer on 1 ml of clear supernatant diluted to 10 ml with buffer (Lab India 3200). Then, calculate the quantity of each active component in each mixture. Ct stands for total drug concentration. Cf stands for free drug concentration.

The Yield of Proliposomes

After the proliposome powders were completely dried, they were collected and carefully weighed.

Using the formula, the yield of proliposomes was estimated.

$$Percentage\ yield = \frac{Total\ weight\ of\ proliposomes}{\text{total weight of drug} + \text{weight of added materials}} \times 100$$

Characterization of Gel

The following gel base characteristics were investigated for both plain gel and gel containing proliposomes [20].

Physical Appearance

All proliposomal gel formulations were visually evaluated for transparency, colour, texture, grittiness, greasiness,

stickiness, smoothness, stiffness, tactiness, and clarity. These were determined in a room with a black-and-white background.

The pH of Formulation

The pH of the gel was measured using a digital pH metre (Lab India SAB 5000), with the glass electrode entirely immersed in the gel structure. For each formulation, the pH values were tested three times (F1-F9).

Rheological Properties

The rheological characteristics of the generated gels were measured using a Brookfield viscometer. The spindle was inserted in the sample container of the Brookfield viscometer after the gel sample was placed in it. The spindle was turning at a rate of 100 revolutions per minute. All rheological experiments were carried out at room temperature. The viscosity was measured in triplicate. The viscosity of carbopol gel at 1%, 2%, and 3% was tested, and the optimal formulation was identified.

Drug Content

A gel containing 10 mg of ketoconazole was dissolved in phosphate buffer (pH 7.4) and tested using a UV-Vis Spectrophotometer at 315 nm to determine its drug concentration [21].

***In-vitro* Studies**

Franz diffusion cells were used to test drug release in vitro. The semipermeable membrane was put between the donor and receptor chambers of the diffusion cell. 30ml of freshly produced 7.4 pH phosphate buffer was added to the receiver chamber. A 1 gm proliposomal gel was placed on a semi-permeable membrane. The Franz diffusion cell was placed above the magnetic stirrer at 500rpm and 3710C, and the temperature was kept constant. On a regular basis, 5ml of sample was extracted and replaced with fresh buffer. The extracted samples were diluted on a regular basis, and the drug concentration was evaluated using a UV visible spectrophotometer (Lab India 3200) set to 3200 nm.

RESULTS AND DISCUSSION

In a preformulation investigation, the optimal amounts of mannitol, phospholipid, and cholesterol were found in order to produce stable liposomes without aggregation, fusion, or sedimentation. Using the thin-film hydration approach, ketoconazole proliposomes were created, and the method was shown to be suitable for the creation of non-aggregating liposomes. It was discovered that the amounts of mannitol, phospholipid, and cholesterol are crucial for the production and stability of proliposomes. For optimal proliposome function, the vesicle size and size distribution of liposomes are the most critical parameters to monitor during proliposome synthesis. Several studies have shown the influence of liposome size on drug release and skin deposition.

With regard to the size of liposome vesicles, a positive association was discovered between phospholipid and cholesterol. Thus, it was revealed that an increase in phospholipid and cholesterol concentration increases vesicle size. A digital microscope (Metzer, India) and water were used to examine a proliposomal sample. The development of a vesicle was then detected within the liposomal dispersion. Count and distribution results for average vesicle size and distribution were computed. In the case of liposomes, entrapment efficiency is a crucial characteristic since it significantly impacts drug release and skin deposition. Results reveal that the efficiency of phospholipid and cholesterol entrapment has increased as concentration has increased. In this investigation, the observed entrapment efficiency for all batches of Ketoconazole proliposome formulation ranged from 85.12 to 96.5 percent, as shown in table no. 2. The Ketoconazole proliposomal formulations F1-F9 with the largest vesicle size and highest entrapment efficiency were chosen for further testing.

At various drug-to-phospholipid ratios, the ketoconazole concentration in proliposomes ranged between 86.4% and 96.8%. Based on the aforementioned findings, it was determined that formulations F4, F1, F5, and F6 had the highest concentrations of medication.

Table 2: Average Particle Size distribution of Proliposomes.

Sr.No.	Formulation Code	Avg particle Size (nm)	Drug Content (%)	Percentage Yield (%)	Entrapment Efficiency (%)
1	F1	5.34±0.023	95.03±0.543	93.4±0.324	94.9±0.244
2	F2	4.43±0.123	86.4±0.734	90.7±0.534	85.12±1.48
3	F3	2.65±0.076	93.7±0.664	89.5±0.654	91.02±0.613
4	F4	6.06±0.012	96.8±0.249	95.4±0.123	96.5±0.205
5	F5	4.34±0.231	94.7±0.984	94.3±0.221	92.7±0.249
6	F6	5.12±0.167	94.8±0.860	94.8±0.212	94.1±0.509
7	F7	3.21±0.221	92.4±1.70	88.7±0.321	88.1±2.19
8	F8	2.69±0.148	90.6±0.748	89.2±0.817	89.2±0.817
9	F9	2.34±0.321	87.5±0.953	86.5±0.265	86.02±2.90

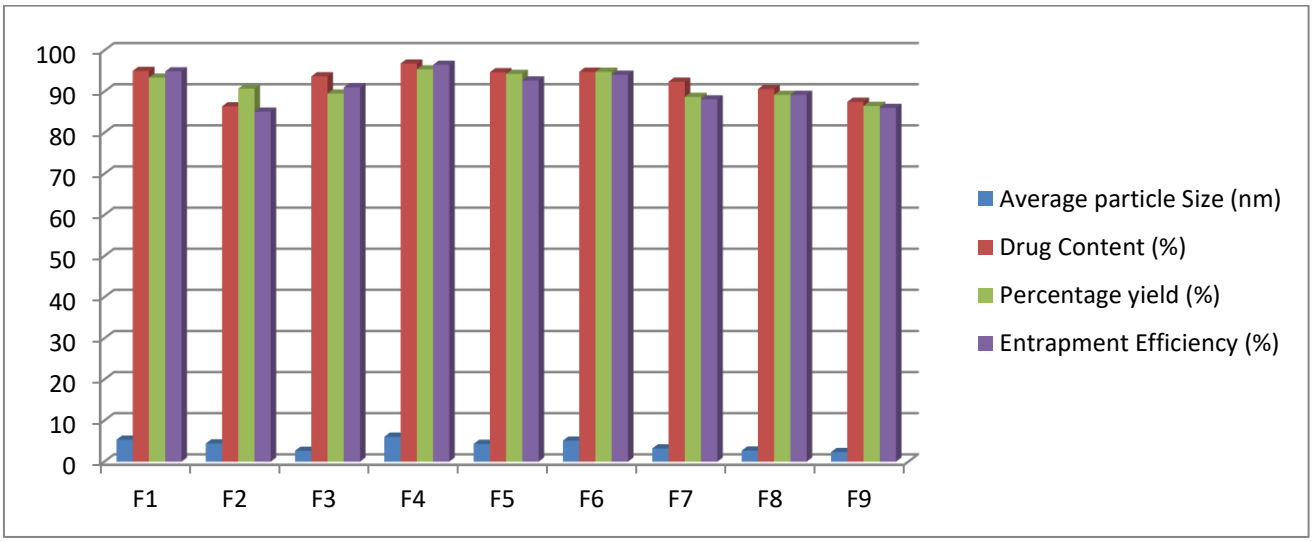

Figure 1: Average Particle Size Distribution, Percent Drug, Entrapment Efficiency, Percent Yield for all formulations of Proliposomes.

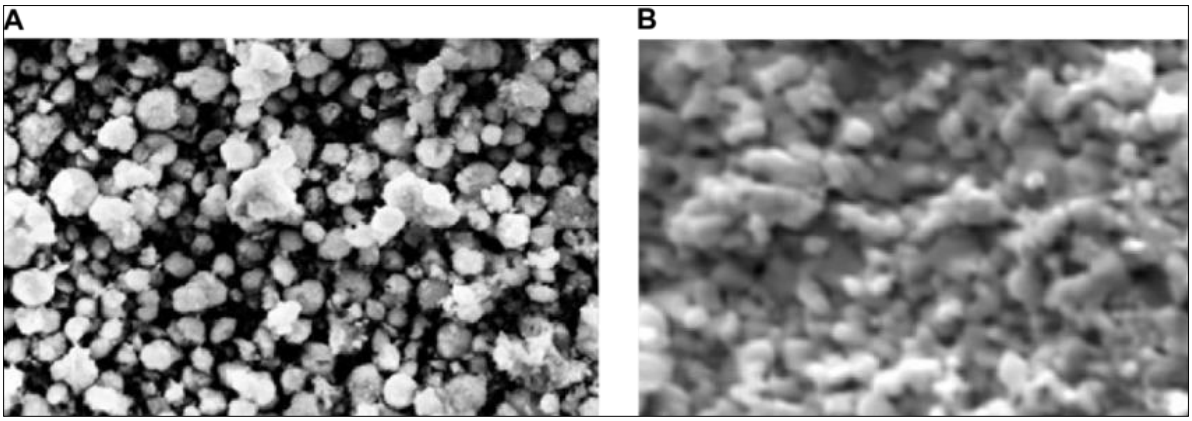

Figure 2: a) SEM of Prepared Liposomes, b) SEM of Gel Loaded with Liposomes

The content of phospholipids was discovered to boost the percentage yield of formulations. As the drug-to-phospholipid ratio in proliposomes was changed, it was found that the percentage yield results of different formulations ranged from 86.5% 0.265% to 95.4% 0.2210%.

Viscosity Measurement

According to rheological studies, 2% carbopol gel outperformed 1% and 3% carbopol gels in terms of rheological properties. As a result, 2% carbopol was used to prepare the proliposomal gel. The gel's viscosity was tested using a Brookfield viscometer. At 100 rpm, the viscosity of proliposomal gel was 1156 cps.

pH Measurement

The pH of the produced product mirrored that of human skin, making it more acceptable. Therefore, the Proliposomal gel formulation was appropriate for topical administration. The pH values of the produced proliposomal gels fell between 5.5 and 5.8.

Scanning Electron Microscopy

The SEM picture of newly formed liposomes reveals the production of spherical-shaped multilamellar vesicles, but the SEM image of gel-loaded liposomes reveals the formation of a drug entrapment layer, which obscures the spherical-shaped vesicles.

Drug Content (%)

The Ketoconazole proliposomal gel that was made had a drug content uniformity of 98.55 percent, which indicates that the medicine was dispersed uniformly throughout the formulation.

***In-vitro* Drug Release**

Ketoconazole was extracted from the gel formulation and then tested for its rate of release in vitro. The results provided strong evidence that the gels were able to keep the drug for longer than expected. The percentage CDR of the proliposomal gel formulation F4 was 80.25 percent, as shown in the figure. Furthermore, this is consistent with the case of Higuchi. It was established that the values of 'n' were more than 0.5 for each and every formulation. This suggests that the release mechanism is similar to non-Fickian diffusion over a longer period of time, which exemplifies the sustained release quality of formulations. It is possible that the presence of phospholipid-rich vesicle domains increased the proportion of lipophilic drug

molecules, such as ketoconazole, that were trapped in a lipid bilayer. This finding suggests that the trapping of ketoconazole in reconstituted liposomes was primarily dependent on lipids. The quantity of the active component that was released may also be controlled by increasing the amount of lipids that are contained inside the proliposome.

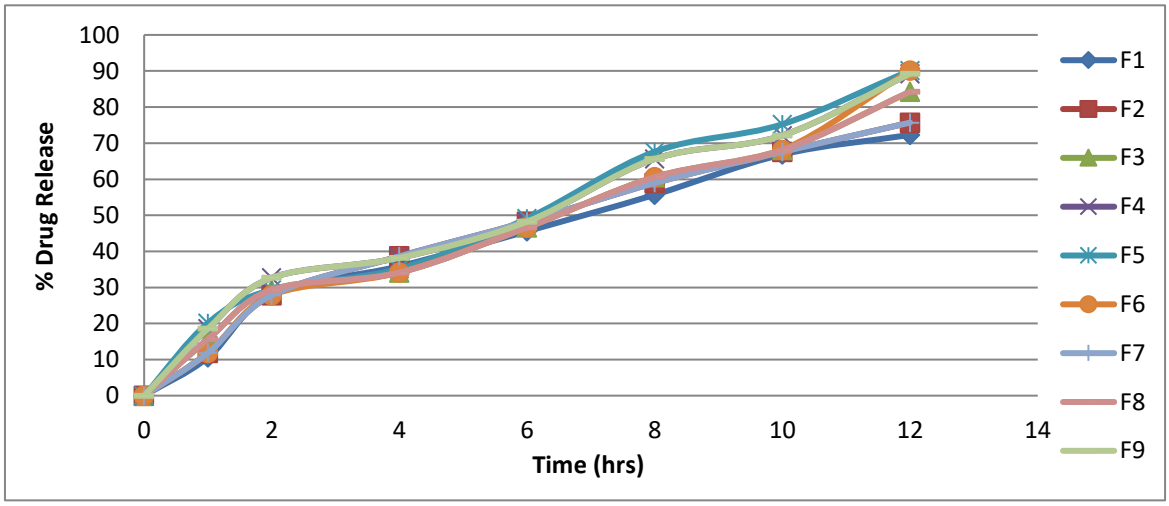

Figure 3: Percent Drug Release for formulations of Proliposomes.

CONCLUSION

In conclusion, a proliposomal drug delivery system can keep ketoconazole in the body for a long time. As the most important part of the liposomal system, phospholipids can be easily mixed with the lipids in the skin and still have the right amount of water to improve drug permeation. The effect of increasing permeation was helped by the fusion of lipid vesicles with skin. It was found that the phospholipid has a big effect on the lipid matrix of the stratum corneum, causing a change in the structure of the lipids between the cells and making penetration easier. So, if the concentration of phospholipids went up, the drug would move through the skin faster after it was put on the skin. The fact that the proliposome granules can flow easily will help when they are made into a solid dosage form. In-vitro studies showed that ketoconazole got through the skin better and stayed in the body longer. This was because the drugs in proliposomes were lipo-solubilized, which helped create the depot effect. The numbers prove that.

Acknowledgment: The author was grateful to the principal and management for giving him the chemicals and space he needed to do his research.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.