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RESEARCH ARTICLE

TOLNAFTATE LOADED LIPOSOMES - DESIGN, AND *IN-VITRO* EVALUATION

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Abstract

Objectives: Liposomes are colloidal particles formed as concentric bimolecular layers that are capable of encapsulating drugs. Liposomes have the potential for extending the duration of action for days or months. Tolnaftate is used as the topical antifungal agent. The purpose of this study was to provide the delivery of the topical drug at a sustained rate across intact skin to improve bioavailability.
Methods: In present study, four different liposomes formulations of Tolnaftate were prepared by ethanol (solvent) injection method by varying the concentrations of phospholipids. The prepared liposomes were characterized for size, shape, entrapment efficiency, zeta potential, *in-vitro* drug release.
Results: The % entrapment efficiency was found to be in the range of 90.24, 90.75, and 90.75%. The % entrapment efficiency of optimized batch LS4 was found to be 90.74 %. An *in vitro* drug release of about 82.114 % in 10 h was observed from optimum formulation of batch LS4.
Conclusion: Based on different parameters like particle size, entrapment efficiency, drug release formulations of batch LS4 was selected as best formulation. Study concludes that Tolnaftate liposomes have potential for providing sustained delivery of drug.
Keywords: Entrapment efficiency, *in-vitro* drug release, liposomes, phospholipid, Tolnaftate, zeta potential.

INTRODUCTION

At present scenario liposome technology is one of the fastest growing scientific field contributing to different types of areas such as drug delivery, cosmetics, nanotechhnology *etc*[1]. The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. Liposomes are concentric bleeder vesicles containing aqueous volume entirely enclosed by a membraneous lipid bilayer[2]. These membranes are usually made of phospholipids, which are molecules that have a hydrophilic head group and a hydrophobic tail group. The head is attracted to water, while the tail, is made of a long hydrocarbon chain, and is repelled by water. Liposomes can be filled with drugs for the treatment of different diseases[3].

Liposomes contains several advantageous characteristics such as ability to incorporate not only water soluble but also lipid soluble agents, specific targeting to the required site in the body and versatility in terms of fluidity, size, charge and number of lamellae. Cholesterol is added to impart different properties like increasing micro viscosity of the bilayer, reducing permeability of the membrane to water soluble molecules, stabilizing the membrane and increasing rigidity of the vesicle[4]. Tolnaftate is a synthetic thio carbamate that is used as the topical antifungal agent. It inhibits the squalene epoxidase enzyme[5]. It is used in the treatment of fungal conditions such as jock itch, athlete's foot and ringworm. Tolnaftate is only active by topical application and inactive when used via oral and intraperitoneal routes[6].

The objective of the present work was to study the preparation, and evaluation of Tolnaftate loaded liposomes in order to increase the release, stability and patient compliance[6].

MATERIALS AND METHODS

Tolnaftate was a gift sample from Green life Pharmaceuticals Ltd. Phospholipid was obtained from Brawn Laboratories, and Sodium alginate from Emzor Pharmaceuticals. Stearic acid and Calcium chloride were obtained from Sewell Pharmaceuticals ltd,

Nigeria. All other chemicals used were of analytical grade.

Tolnaftate liposomes were prepared by ethanol (solvent) injection method. The lipid, cholesterol, stearic acid and lipid soluble component, drug (25 mg) were dissolve in ethanol and injected in to 10 ml preheated distilled water at 55-65°C with continuous stirring at 500 rpm using magnetic stirrer. The solvent was evaporated by heating so as to obtain drug loaded liposomes[7].

Table 1: Composition of Tolnaftate liposomes.

Formulation code	Phospholipid (mg)	Cholesterol (mg)	Sodium alginate (ml)	Calcium chloride (ml)	Stearic acid (mg)
LS1	60	40	10	25	10
LS2	50	50	10	25	10
LS3	40	40	10	25	10
LS4	30	50	10	25	10

Characterization of liposomes

Particle size analysis and surface morphology

The particle size of Tolnaftate liposomes was determined by optical microscopy. All the prepared batches of Liposome's were viewed under microscope to study their size. Size of liposomal vesicles was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles were determined. The surface morphology was studied by scanning electron microscopy[8,9].

Measurement of Zeta potential

Zeta potential of the liposomes was measured using electrophoretic light scattering by a Malvern Zetasizer Nano ZS. The measurement was performed at 25°C after appropriate dilution with distilled water. All of the measurements were repeated three times[10,11].

Drug entrapment efficiency of liposomes

Entrapment efficiency of Tolnaftate liposomes was determined by centrifugation method. Aliquots (1 ml) of liposomal dispersion were subjected to centrifugation on a laboratory centrifuge at 3500 rpm for a period of 90 min. The clear supernatants were removed carefully to separate non-entrapped Tolnaftate and absorbance recorded at 256 nm[12,13].

***In vitro* drug release study**

The release studies were carried out in diffusion cell having 10 ml capacity. 10 ml phosphate buffer pH 7.4 was placed in diffusion cell. The diffusion cell contained a magnetic bed and the medium was equilibrated at 37±5°C. Dialysis membrane was taken and placed on the diffusion cell. After separation of non-entrapped Tolnaftate liposomes dispersion was filled in the dialysis membrane[14,15]. The dialysis membrane containing the sample was suspended in the medium. Aliquots were withdrawn (1 ml) at specific intervals, filtered, diluted with phosphate buffer and the absorbance was taken at 256 nm. Then the apparatus was immediately replenished with same quantity of fresh phosphate buffer pH 7.4 medium.

Statistical analysis

Experimental results were expressed as mean±SD. Student's *t*-test and one-way analysis of variance (ANOVA) were applied to check significant differences in drug release from different formulations. Differences were considered to be statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

SEM image of Tolnaftate loaded liposomes (Batch LS4) is shown in Figure 1. The image indicates that liposomes of spherical shape were formed by the method employed to prepare them. Spherical and rod-shaped particles effectively adhere to cells. Hence, there is a greater probability for Tolnaftate loaded liposomes to adhere to cells. Vesicle size plays an important role with respect to permeation of liposomes through different membrane barriers. The vesicle size-range of all the Tolnaftate liposomes formulations was found to be 325.83 to 400.25 nm. It confirms the normal size distribution of the vesicles.

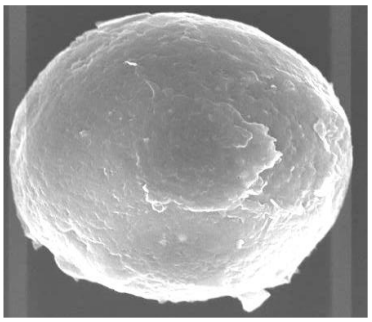

Figure 1: SEM of Tolnaftate liposomes of batch LS4.

The reproducibility of the liposomal formulation with respect to size was confirmed by preparing the formulations three times, but the statistical analysis was avoided as the particle size data was highly reproducible each time.

Table 2: Evaluation parameters of Tolnaftate liposomes.

Formulation code	Zeta potential (mV)	Vesicle size (nm)	% Entrapment efficiency
LS1	-18.4±0.87	325.83±0.15	90.24±0.08
LS2	-17.3±0.24	340.23±0.63	90.37±0.11
LS3	-16.5±0.41	375.18±0.48	90.58±0.31
LS4	-15.4±0.09	400.25±0.51	90.74±0.52

Mean ± SD, N=3

Higher vesicle size of Batch LS4 of liposomes was observed, it may be due to partial aggregation. The % entrapment efficiency was found to be in the range of 90.24, 90.75, and 90.75%. The % entrapment efficiency of optimized batch LS4 was found to be

90.74%. Figure 4 shows *in vitro* drug release profile. The release characteristic could be attributed to the fact that Tolnaftate was trapped by the lipid, and therefore, Tolnaftate might get released gradually from the lipid vesicles.

In a study of 10 hrs, maximum release 82.114% was shown by optimized batch LS4, while minimum 51.4% drug release was shown by liposomes of batch LS3. It was observed that increase in the lipid concentration delays the drug release due to increased particle size and reduced surface area available for drug release.

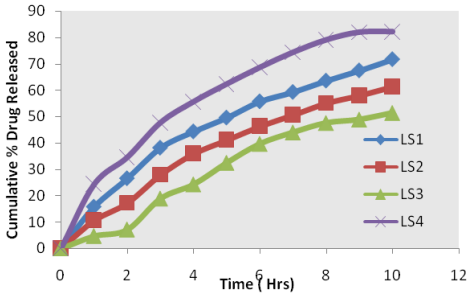

Figure 2: *In-vitro* drug release profile of Tolnaftate liposomes.

CONCLUSIONS

The present study has been a satisfactory attempt to formulate and evaluate liposome of Tolnaftate with a providing sustained delivery of drug. Ethanol (solvent) injection method was used to prepare liposome employing ethanol as solvents to dissolve the drug and the excipients. The prepared formulations were characterized for their particle size, morphology, drug entrapment, and *in-vitro* drug release studies. Almost all the formulations showed fairly acceptable values for all the parameters evaluated. The formulations showed good drug entrapment and *in vitro* released. The surface morphology of the prepared liposome was studied using scanning electron microscopy. From the SEM study it was conclude that prepared liposomes were spherical in shape. Based on different parameters like particle size, entrapment efficiency, drug release formulations of batch LS4 was selected as best formulation.

AUTHOR'S CONTRIBUTION

John DF: writing, review, and editing, methodology. **Yunus AA:** writing, review, and editing. **Chigbo UJ:** writing, review. **Paul US:** formal analysis, writing, review. **Ikenna E:** writing, review, and editing, investigation, data curation, conceptualization. All authors read and approved the manuscript.

CONFLICT OF INTERESTS

None to declare.

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