

TOLNAFTATE LOADED LIPOSOMES- DESIGN, AND IN-VITRO EVALUATION

ABSTRACT-

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. 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. An in vitro drug release of about 82.114 % in 10 h was observed from optimum formulation of batch LS4.

Key words: Tolnaftate, liposomes, phospholipid, entrapment efficiency, zeta potential, in-vitro drug release.

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, nanotechnology etc¹.

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 membranous lipid bilayer². 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, is repelled by water. Liposomes can be filled with drugs to for the treatment of different diseases³.

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 vesicle⁴.

Tolnaftate is a synthetic thio carbamate that is used as the topical antifungal agent. It inhibits the squalene epoxidase enzyme⁵. 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 routes⁶.

Preparation of liposomes

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 55⁰ - 65⁰C with continuous stirring at 500 rpm using magnetic stirrer. The solvent was evaporated by heating so as to obtain drug loaded liposomes⁷.

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^{8,9}**

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.

Measurement of Zeta potential^{10,11}

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.

Drug entrapment efficiency of liposomes^{12,13}

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.

$$\% \text{ Drug Entrapped (PDE)} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug}} \times 100$$

In vitro drug release study^{14,15}

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. 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.

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=

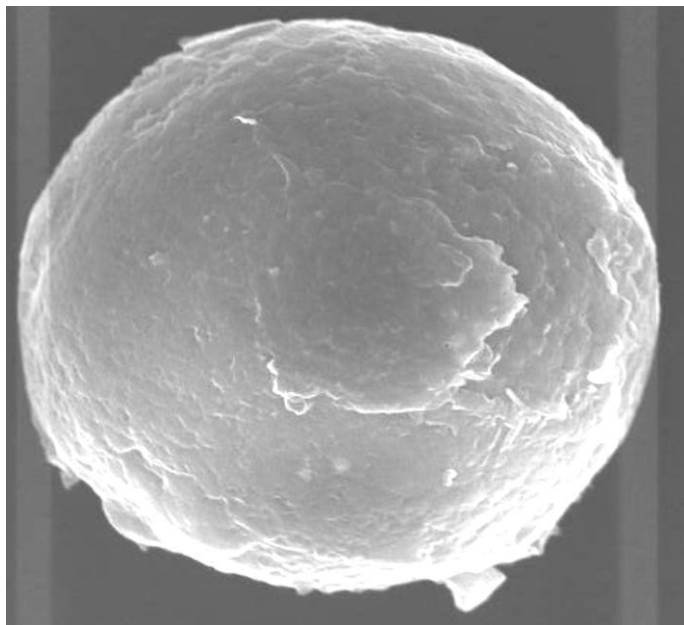


Fig 1: SEM of Tolnaftate liposomes of batch LS4

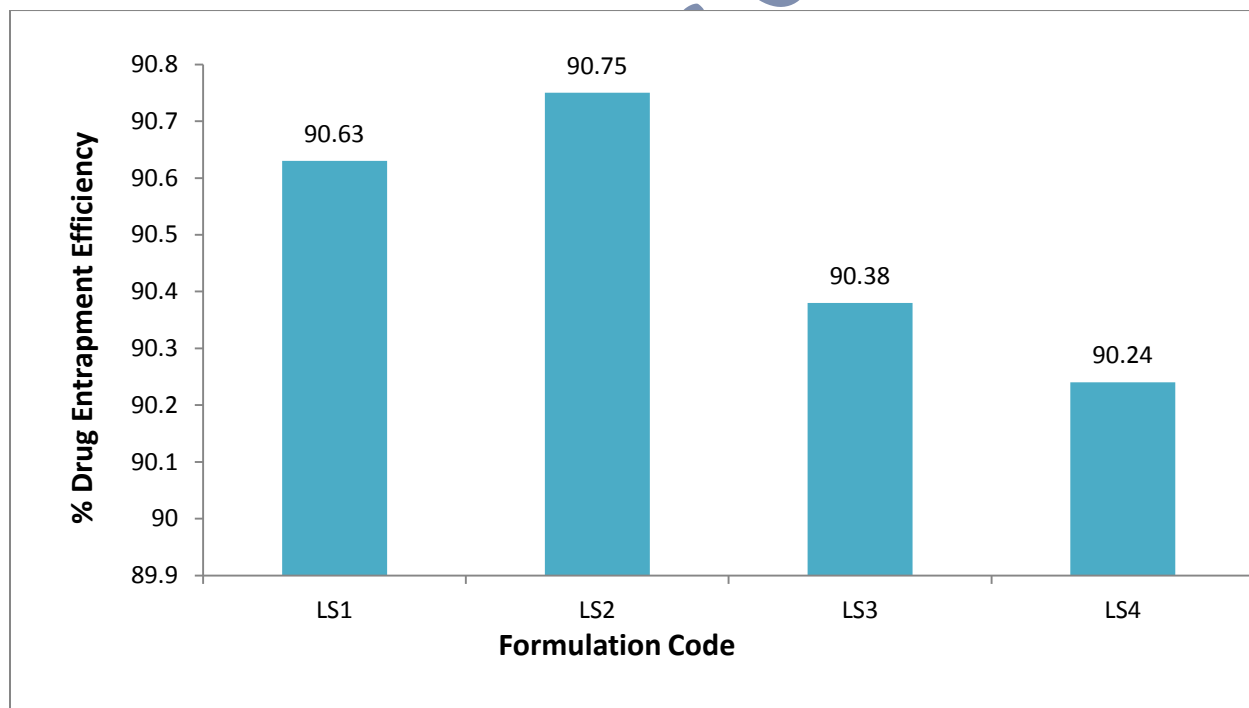


Fig-2: Entrapment efficiency of different Tolnaftate liposomes formulations

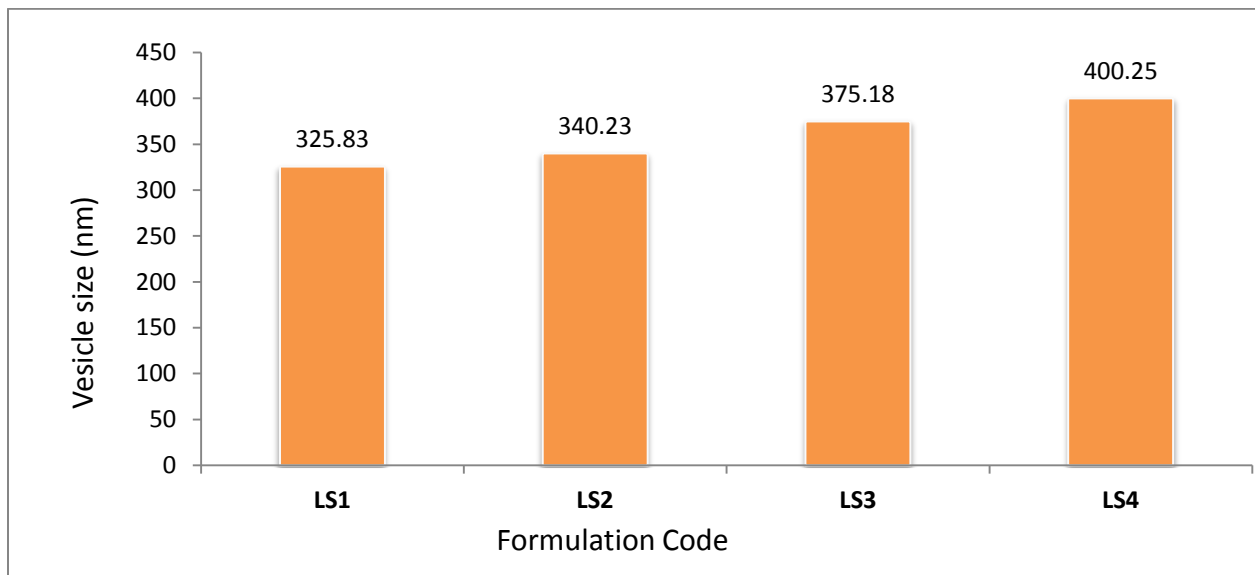


Fig-3: Vesicle size of different Tolnaftate liposomes formulations

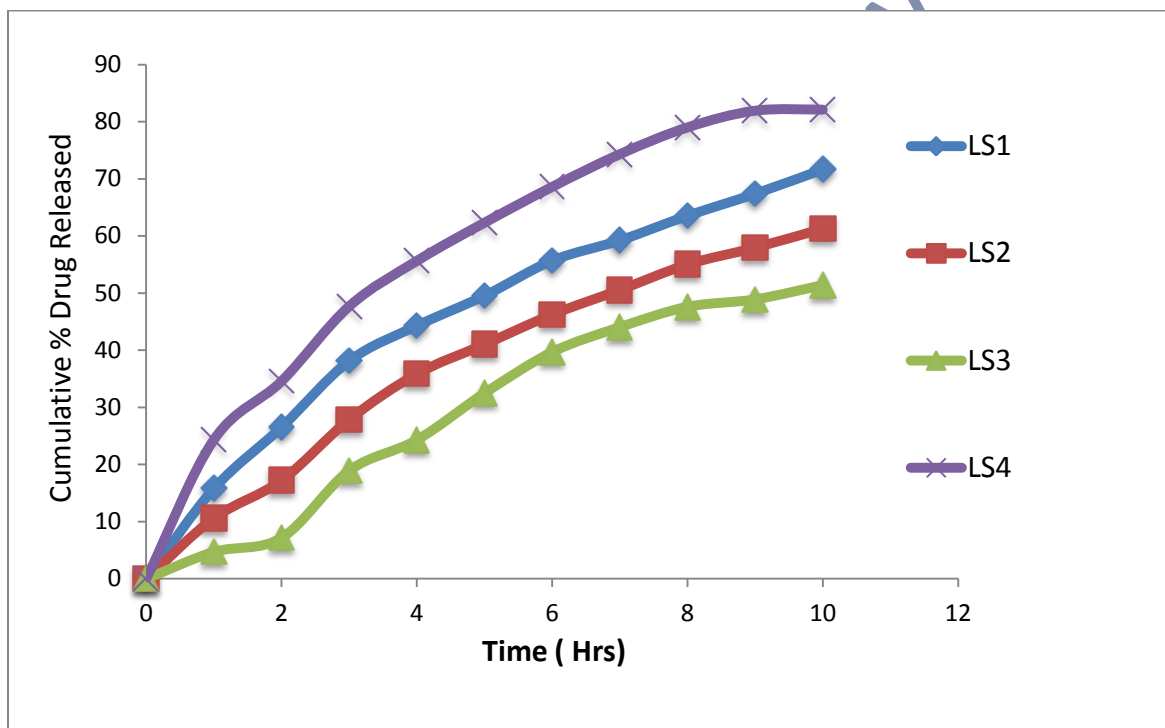


Fig-2: *In-vitro* drug release profile of Tolnaftate liposomes

RESULTS AND DISCUSSION:

SEM image of Tolnaftate loaded liposomes (Batch LS4) is shown in Fig. 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. 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. 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 90.75 %. The % entrapment efficiency of optimized batch LS4 was found to be 90.74 %.

Figure 2 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.

CONCLUSION:

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 concluded 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.

CONFLICT OF INTERESTS

The authors who have taken part in this study declared that they don't have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

REFERENCES-

1. Allen TM. Long-circulating (sterically stabilized) liposomes for targeted drug delivery. *Trends Pharmacol Sci*, 1994;15:215–20.
2. Bangham AD, Standish M, Klatins JC. Diffusion of univalent ions across the lamella of swollen phospholipids, *Journal of Molecular Biology*, 1995; 13: 238-252.
3. Ertel I, Nesbit M, Hammond D, Kleiner J, Sather H, Effective dose of L-asparaginase for induction of remission in previously treated children with acute lymphoblastic leukemia, A report from Children Cancer study Group. *Cancer research*, 1979; 39 : 893-3896.
4. Gasper MM., Perez Soler R, Cruz MEM, Biological Characterization of L-asparaginase liposomal formulation, *Cancer Chemotherapy and Pharmacology*, 1996; 38: 373-377.
5. Ryder NS, Frank I, Dupont MC. Ergosterol biosynthesis inhibition by the thiocarbamate antifungal agents tolinaftate and tolciclate. *Antimicrob. Agents Chemother*, 1986, 29 (5): 858–60.
6. Crawford F, Hart R, Bell-Syer S, Torgerson D, Young P, Russell I. Topical treatments for fungal infections of the skin and nails of the foot. *In: The Cochrane Library*, 2003, 1, 84.
7. Jorge JCS, Perez Soler R., Moruis JG, Cruz MEM, Liposomal mitoyl L-asparaginase: characterization and biological activity , *Cancer Chemotherapy and Pharmacology*, 1994; 34 : 230-234.
8. Morris F, Gerald KL, The introduction of enzymes into cells by means of liposomes. *Journal of Lipid Research*, 1978; 19: 289-303.
9. Ritger PL, Peppas NA. A simple equation for description of solute release I: fiction and non – fiction release from non– swellable devices in the form of slabs , spheres , cylinders or discs, *Journal of Control Release*, 1985; 5:23-35.

10. Vingerhoeds MH, Haisma HJ, Van Muijen M., VandeRiji. Crommelin DJA., Storm G. A new application of liposomes in the cancer therapy FEBS LEH, 1993; 336: 485-490.
11. M. Kokabi, M. Sirousazar, ZM Hassan. PVA-clay nanocomposite hydrogels for wound dressing. *European Polymer Journal*, 2007 43: 773-781.
12. Sauvez F, Drouin DS, Attia M, Bertheux H, Forster R. Cutaneously applied 4-hydroxytamoxifen is not carcinogenic in female rats. *Carcinogenesis*, 1999, 20: 843-850.
13. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the swollen phospholipids. *J Mol Biol*, 1965, 13: 238-252.
14. Jordan VC, Tamoxifen: toxicities and drug resistance during the treatment and prevention of breast cancer. *Annu Rev Pharmacol Toxicol*, 1995, 35: 195-211.
15. Liang HF, Yang TF, Huang CT, Chenc MC, Sung HW. Preparation of nanoparticles composed of poly (gamma-glutamic acid)-poly (lactide) block copolymers and evaluation of their uptake by HepG2 cells. *J.Control. Release*. 2005; 105:213-215.

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