Indian Journal of Medical and Allied Research

Volume 11, Issue 3, Dec 2022 pp 1-5. **www.ijmar.in** ISSN: 2278-0890

Assessment Of Antifungal Drug-Loaded Liposomes

Swadesh Nayak[1] *, Rohit Saraswat [2]

1. Research Scholar Sunrise University, Alwar, Rajasthan, India
2. Research Guide Sunrise University, Alwar, Rajasthan, India

Submitted: 03-12-2022 Accepted: 19-12-2022

ABSTRACT

Antifungal liposome was prepared by thin film method using a varying ratio of cholesterol, soya lecithin, and stearyl amine. The amount of soya lecithin was kept constant in all batches. Stearyl amine was used as a charge-imparting agent. The effect of formulation variables on entrapment efficiency, particle size, surface charge, and drug release behavior was studied. Encapsulation efficiency of oxiconazole -loaded liposomes was determined by the centrifugation method. The inclusion of positively charged surfactants such as stearyl amine significantly increases the entrapment efficiency of oxiconazole into the liposomes. The LF8 formulation showed the highest drug-loading capacity of 94.34%±3.03 when compared with the other formulations. The cumulative amount of drug release from Bifonazole liposome was 57.25%-93.21%, after 24 hours. The release of oxiconazole from liposomes changed with phospholipid and cholesterol ratio (LF1-LF6). In conclusion, a stable oxiconazole-loaded liposomal formulation was successfully formulated for the topical administration.

Keywords: Oxiconazole, Hydrogel, Liposome, soya lecithin and fungal infection Drug permeability, Stability studies

INTRODUCTION

Fungal infection of the skin is nowadays one of the common dermatological problems. The physicians have a wide choice of treatment from solid dosage to semisolid dosage form and liquid dosage formulation[1-3]. Among the topical formulations, liposomes have been widely investigated in both cosmetics and pharmaceuticals. Liposomes and other types of lipid vesicles have been employed for many years in the cosmetic industry and have been widely investigated for their potential to enhance transdermal absorption and local drug delivery of a variety of agents. In many cases, skin penetration is enhanced when liposomal formulations are used compared with formulations containing free-drug. Despite years of research, the mechanism(s) responsible for liposome-enhanced dermal absorption of drugs by intact skin has not been established. Liposomes of different sizes and different properties can be made by varying the lipid composition, the manufacturing process, and the inclusion of surfactants and these different factors serve to modify the transdermal transport of the enclosed drugs. Oxiconazole liposomes were prepared by the thin-film hydration method. Prepared liposomes were evaluated for surface morphology, drug content, encapsulation efficiency, *in-vitro* drug release behavior, *in-vitro* release kinetics study, and short-term stability studies[4-8].

Encapsulation Efficiency (%EE):

The encapsulation efficiency of oxiconazole-loaded liposomes was determined by the centrifugation method. Entrapment efficiency is expressed as the percentage of the total amount of drug used initially. The entrapment efficiency of all liposomes was in the range of 48.85% ± 3.74 to 94.34 % ± 3.09. Table 1 shows that Bifonazole entrapment efficiency varied with lipid composition and cholesterol content. The results show that the percentage entrapment efficiency of oxiconazole increased by increasing the cholesterol content. The percentage entrapment efficiency of oxiconazole-loaded liposomes (LF1) containing soya lecithin: cholesterol in the ratio 1:0.15 was 48.85%, whereas the entrapment efficiency was higher (84.27%) in liposome LF4, prepared with soya lecithin: cholesterol in the ratio 1.0:0.6. Further increase in entrapment efficiency was not recorded for liposomes composed of soya lecithin: cholesterol in the ratio 1:0.8 and 1:1. Cholesterol molecules are placed between the adjacent phospholipid molecules in the liposomal bilayer and hence occupy some space and compete with oxiconazole for incorporation into the bilayer. Additionally, cholesterol makes the bilayer more rigid, which makes the incorporation of the oxiconazole molecules harder. In this study, the 1:0.8 and 1:1 molar ratio of cholesterol to the phospholipid caused a dramatic decrease in encapsulation efficiency, which is speculated to be due to a defect in the regular linear structure of the liposomal bilayer. Disruption of the regular linear structure of the liposomal bilayer causes a

prominent decrease in encapsulation efficiency for both lipophilic and hydrophilic molecules. A potential benefit of cholesterol in liposomes is that although the encapsulation efficiency could be decreased, the escape or release of drug molecules from the liposomal membrane could also be decreased due to an increase in membrane rigidity. The inclusion of positively charged surfactants such as stearyl amine tends to increase the inter lamellar repeat distances between successive bilayers in the liposome, swelling the structure with the greatest proportion of the aqueous phase. These effects lead to a greater overall entrapped volume. Among the liposome formulation containing Stearylamine, formulation LF8 (containing a 1.0:0.6:0.4 ratio of soya lecithin: cholesterol: Stearyl amine) showed the highest percentage of entrapment (94.34%). Further increase in entrapment efficiency was not recorded for liposomes composed of soya lecithin: cholesterol: Stearylamine in the ratio 1:0.6:0.6.

Table 1: Formulation design of oxiconazole-loaded liposome

Formulation	% Entrapment efficiency (n=3, ±SD)
LF1	48.85±3.74
LF2	57.39±2.49
LF3	65.20±3.62
LF4	84.27±3.36
LF5	78.59±2.90
LF6	71.43±3.67
LF7	89.32±3.41
LF8	94.34±3.03
LF9	90.22±3.19

Determination of particle size and zeta potential[9-11]:

Liposomes were developed through the thin-film hydration method, which is the most common method for liposome preparation. Since lipidic composition and concentration could have a significant impact on developing a therapeutically efficient liposomal carrier system, we examined different parameters such as an increase in cholesterol ratio, and the presence of more unsaturations in the phospholipid's acyl chain. Since cholesterol has a rigid ring and an ultra-smooth face in its structure, the squeezing process has most likely taken place by allowing the lipids to turn quickly at the extremities. This leads to lipids of high lateral mobility, as well as packed acyl chains. Consequently, this contributes to the variation of sizes since the packing factor affects the intensity and duration of van der Waals forces present in liposomes. In addition, by increasing the concentration of Cholesterol in the formulation, the structural interaction propitiates vesicular aggregations which may lead to slightly bigger liposomes. The results of a study demonstrated that the size of the drug-loaded liposome increased significantly from 68.0 nm to 164.6 nm when the cholesterol concentration was increased. Formulation LF1 showed the lowest particle (68.0 nm) and formulation LF6 showed the highest particle size (164.6 nm).

The effect of stearylamine concentration on the particle size of liposomes was also studied. With the increase in the stearyl amine concentration, the particle size increased. It may attribute the fact the presence of high stearylamine to the inclusion of a charge inducer in liposomes, which modified the spacing between the adjacent bilayers. The results of a study demonstrated that the size of the drug-loaded liposome increased significantly from 137.4 nm to 195.0 nm when stearylamine was added in the formulation. It has been previously shown that drug delivery to deeper layers of skin requires liposomal particle size smaller than 600 nm. Vesicles larger than 600 nm tend to stay on the stratum corneum and may dry to form a lipid layer on the skin. Vesicles smaller than 300 nm were able to deliver their payload to some extent into deeper layers of skin. All the formulations used in this study showed a polydispersity index below 0.3, indicating a good homogeneity for the prepared liposomes.

Zeta potential was measured by dynamic light scattering at room temperature. Liposome dispersions were diluted with phosphate buffer before the measurement to adjust the intensity. The zeta potential values of liposomal formulations (LF1-LF6) were found to be negative and in the range of -18.6 mV to -28.1 mV owing to the net charge of the lipid content in the nano-formulations. The surface charge of liposome LF6-LF9 was found to be positive and in the range of 21.9 mV to 26.2 mV. In general, charged liposomes were more stable against aggregation and fusion than uncharged liposomes. The magnitude of zeta potential in all prepared formulations is sufficiently high to prevent coagulation and provide stability for the vesicles. Results of particle size, PDI and zeta potential are shown in table 2.

Table 2: Evaluation of Bifonazole-loaded liposome

Formulation	Particle size (nm)	PDI	Zeta potential (mV)
LF1	68.0	0.190	-23.4
LF2	122.1	0.231	-20.5
LF3	129.0	0.229	-23.1
LF4	134.5	0.204	-24.0
LF5	147.2	0.166	-26.6
LF6	164.6	0.199	-18.6
LF7	137.4	0.237	26.9
LF8	182.6	0.219	29.5
LF9	195.0	0.213	34.22

Indian Journal of Medical and Allied Research

Volume 11, Issue 3, Dec 2022 pp 1-5. **www.ijmar.in** ISSN: 2278-0890

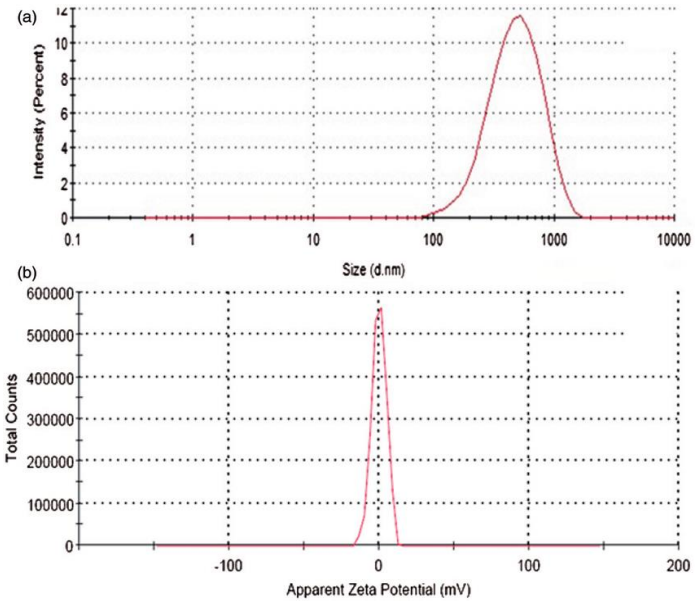

Figure 1: Particle size distribution of Oxiconazole loaded liposome

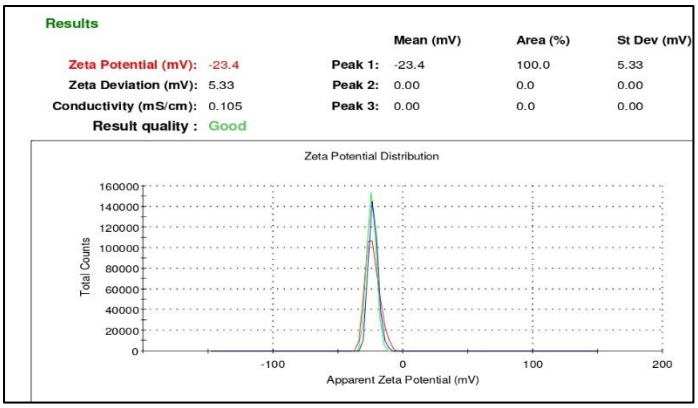

Figure 2: Zeta potential determination of oxiconazole-loaded liposome

***In-vitro* drug release studies:**

The drug release profiles of oxiconazole liposomes are displayed in figure 9. Optimal conditions for drug release were determined from the equilibrium solubility of oxiconazole. Oxiconazole has good solubility in phosphate buffer solution (pH 6.8) containing 1.0% Tween 80. The cumulative drug release from Bifonazole liposome was 57.25%-93.21%, after 24 hours. As seen in figure 9, the release of oxiconazole from liposomes changed with phospholipid and cholesterol ratio (LF1-LF6). Drug release was delayed as molar ratio of soya lecithin increased in the formulation ($p < 0.05$). This might be due to the interaction of oxiconazole with the large surface of the lipid bilayer membrane of liposome and soya lecithin layer thickness and fluidity of the membrane. The release from liposomes was not completed during the test and the amount of released drug decreased oxiconazole with the increase of cholesterol content in the liposomes (LF1-LF6). Oxiconazole release from the LF6 formulation, which contained the highest molar ratio of cholesterol, was the lowest among all other formulations (68.42%). High membrane permeability and the stabilizing effect of cholesterol on membrane structure may be the possible reason. As seen in figure 3, when electropositive detergent like Stearylamine was added into the formulation, the electropositive charge of the liposomes increased and thus slows the release of oxiconazole from liposomes. The findings of the present investigation showed that the drug release was slower with the addition of Stearylamine into the liposomal formulations (LF7-LF9).

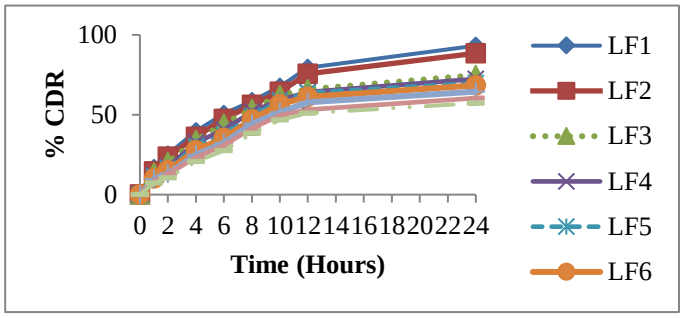

Figure 3: Comparative *in-vitro* drug release profile of oxiconazole liposomes (LF1-LF9)

Drug Release Kinetics: The kinetics of drug release from a liposomal formulation is a critical part of the rational design of drug delivery systems, as it is a major determinant of the efficacy of delivery of the carrier *in-vivo* and the subsequent release of the free drug. An *in-vitro* release profile reveals important information on the structure and behavior of the formulation, possible interactions between the drug and carrier composition, and their influence on the rate and mechanism of drug release. The pattern of the drug release from the oxiconazole liposome was investigated by different kinetic equations. The data of cumulative release were subjected to various kinetics models and results obtained from release kinetics studies were depicted in table 3. The *in-vitro* release profile of the drug from all liposomal gel formulations could be expressed by Higuchi kinetics, as the plots show high linearity (r^2 = 0.990-0.998) in comparison to zero order (r^2 = 0.801-0.896) and first-order kinetics (r^2 = 0.921-0.976). Liposome gel had the highest r^2 for the Higuchi model indicating that the drug release process was controlled by diffusion. This is in agreement with previous reports. The correlation coefficients obtained for the Korsmeyer–Peppas equation were also quite high (0.870 to 0.932). All of the values of the release exponent (n) were higher than 0.85, which corresponds to the super case II release mechanism.

Indian Journal of Medical and Allied Research

Volume 11, Issue 3, Dec 2022 pp 1-5. **www.ijmar.in** ISSN: 2278-0890

Table 3: Drug release kinetics of formulation LFG1 to LFG9

Formulation code	KINETIC MODELS					Model Followed
	Higuchi	Zero order	First order	Korsmeyer-Pappas		
	r^2	r^2	r^2	r^2	n	
LFG1	0.995	0.872	0.956	0.927	0.901	supercase II
LFG2	0.991	0.869	0.973	0.921	0.870	supercase II
LFG3	0.997	0.801	0.934	0.932	0.896	supercase II
LFG4	0.998	0.895	0.921	0.929	0.890	supercase II
LFG5	0.995	0.878	0.976	0.978	0.932	supercase II
LFG6	0.990	0.892	0.968	0.960	0.884	supercase II
LFG7	0.996	0.890	0.970	0.989	0.919	supercase II
LFG8	0.994	0.894	0.938	0.981	0.925	supercase II
LFG9	0.994	0.896	0.938	0.981	0.922	supercase II

r^2=Regression coefficient, **n=** Exponential value

Physical Stability at Storage Condition[12-15]:

The stability of liposomes is an essential requirement concerning its size and efficacy. The stability of Bifonazole loaded liposomes (LF8) was examined over 30 days, 60 days and 90 days in triplicate. Drug content and release profile were monitored and did not display any significant change compared to initial sample (Day 1). Interestingly, significant changes in drug content were not observed up to 90 days of storage. Moreover, there was no significant change in the appearance of the liposome. Thus, the developed liposome formulation was physicochemically stable throughout the stability period. The results of stability studies were shown in table 4.

Table 4: Stability studies for liposome formulation LF8

Days	25°C/60% RH		40°C/75% RH	
	% Drug content	% CDR	% Drug content	% CDR
Initial	101.16±0.60	56.91±0.42	101.16±0.60	56.91±0.42
30	101.12±0.47	56.86±0.11	101.02±0.36	56.54±0.57
60	101.05±0.93	56.42±0.40	100.56±0.71	56.22±0.90
90	100.32±0.35	56.10±0.83	100.18±0.94	56.08±0.48
P value	>0.05	>0.05	>0.05	>0.05

CONCLUSION

Antifungal Drug-Loaded Liposomes:

Liposomes are microscopic vesicles consisting of phospholipid bilayers that enclose aqueous compartments and are utilized as drug-delivery systems for both hydrophilic and lipophilic drugs. Liposomes are one of the most suitable drug-delivery systems to deliver a drug to the target organ and minimize the distribution of the drug to non-target tissues. The importance of liposomes as a drug-delivery vehicle is now becoming more established, and they deliver drugs in a controlled manner when compared with conventional dosage forms. In the present research work, oxiconazole-loaded liposome was prepared by thin film method. Nine different formulations of liposome were prepared by varying ratios of cholesterol, soya lecithin, and stearyl amine. The amount of soya lecithin was kept constant in all batches. The encapsulation efficiency of oxiconazole-loaded liposomes was determined by the centrifugation method. The inclusion of positively charged surfactants such as stearyl amine significantly increases the entrapment efficiency of oxiconazole into the liposomes. The LF8 formulation showed the highest drug-loading capacity when compared with the other formulations. Oxiconazole-loaded liposomal gel showed a prolonged drug release profile. Taken together, these results revealed that the merits of the developed liposomal formulation as an effective and safe treatment of fungal infection among patients justify their potential in strengthening the efficacy and safety of the drug.

REFERENCES

1. Pachuau, L. (2015) 'Recent developments in novel drug delivery systems for wound healing', *Expert Opinion on Drug Delivery*, 12(12), pp. 1895–1909.
2. Amin, S., Rajabnezhad, S. and Kohli, K. (2009) 'Hydrogels as potential drug delivery systems', *Scientific Research and Essay*, 3(11), pp. 1175–1183.
3. Singh Malik D, Mital N, Kaur G. Topical drug delivery systems: a patent review. Expert opinion on therapeutic patents. 2016;26(2):213-28.
4. Joshi M, Butola BS, Saha K. advances in topical drug delivery system: micro to nanofibrous structures. J NanosciNanotechnol 2014; 14:853-67.
5. Esenturk I, Balkan T, Ozhan G, Doşler S, Gungor S, Erdal MS, *et.al.*, Voriconazole incorporated nanofiber formulations for topical application: preparation, characterization, and antifungal activity studies against Candida species. Pharmaceutical development and technology. 2020;25(4):440-53.
6. Garg A, Sharma GS, Goyal AK, Ghosh G, Si SC, Rath G. Recent advances in topical carriers of anti-fungal agents. Heliyon. 2020;6(8): e04663.
7. Ghavaminejad, A., Park, C. H. and Kim, C. S. (2016) 'In Situ Synthesis of Antimicrobial Silver Nanoparticles within Antifouling Zwitterionic Hydrogels by Catecholic Redox Chemistry for Wound Healing Application', *Biomacromolecules*, pp. 1213-1223.
8. Hoffman, A. S. (2012) 'Hydrogels for biomedical applications', *Advanced Drug Delivery Reviews*. Elsevier B.V., 64, pp. 18–23.
9. Harrison, I. P. and Spada, F. (2018) 'Hydrogels for Atopic Dermatitis and Wound Management: A Superior Drug Delivery Vehicle'.
10. Ahsan A, Tian WX, Farooq MA, Khan DH. An overview of hydrogels and their role in transdermal drug delivery. International Journal of Polymeric Materials and Polymeric Biomaterials. 2021;70(8):574-84.
11. Mohan YM, Premkumar T, Lee K, Geckeler KE. Fabrication of silver nanoparticles in hydrogel networks. Macromolecular Rapid Communications. 2006;27(16):1346-54.
12. Nagam SP, Jyothi AN, Poojitha J, Aruna SA, Nadendla RR. A comprehensive review on hydrogels. *Int. J. Curr. Pharm. Res.* 2016;8(1):19-23.

13. Rakesh Yeshaswi, Prathima Srinivas, Sadanandam M. Development and characterization of microemulsion formulations for transdermal delivery of aceclofenac: research. *Int J Drug Formulation Res* 2010; 1:359-86.
14. Gulrez, S. K.; Phillips, G. O.; Al-Assaf, S. Hydrogels: Methods of Preparation, Characterisation and Applications. *J. Adv. Res.* 2011, 5, 117–150.
15. Rowley JA, Madlambayan G, Mooney DJ. Alginate hydrogels as synthetic extracellular matrix materials. Biomaterials. 1999;20(1):45-53.