PROSPECTS
IN PHARMACEUTICAL SCIENCES

Prospects in Pharmaceutical Sciences, 23(1), 102-110
https://prospects.wum.edu.pl/

Original Article

ADVANCED INVASOMAL DELIVERY SYSTEM FOR ENHANCED TOPICAL APPLICATION OF BIFONAZOLE IN FUNGAL INFECTIONS

Shivaji Patil[1]*, Sushil Bhargav[1]

[1] *Department of Pharmaceutical Sciences, Madhav University, Abu Road Pindwara, Dist Sirohi -307026, Rajasthan.*

* Correspondence, e-mail: shivajimpatil1993@gmail.com

Received: 25.11.2024 / Accepted: 23.01.2025 / Published: 01.03.2025

ABSTRACT

The design and development of invasomes containing bifonazole for effective topical fungal treatment was investigated to improve the bioavailability and therapeutic efficacy of this antifungal agent. Invasomes, novel lipid-based vesicular systems, were formulated to enhance skin penetration and provide sustained drug release, thereby increasing the local concentration of bifonazole at the site of infection. The formulation was optimized using response surface methodology (RSM), where various factors such as phosphatidylcholine, terpenes, and ethanol were varied to evaluate their effects on entrapment efficiency, vesicle size, and drug release characteristics. The optimized invasomal formulation demonstrated high entrapment efficiency (~74.12%) and an average vesicle size of 154.45 nm, suitable for effective skin penetration. In vitro release studies revealed sustained drug release from the invasomes, with a slower release profile compared to the plain drug. The optimized formulation exhibited enhanced antifungal activity, suggesting that invasomes could serve as a promising carrier for the topical delivery of bifonazole, offering a potential solution for treating superficial fungal infections with improved efficacy and patient compliance.

KEYWORDS: Invasomes, Bifonazole, Drug Encapsulation, Skin Penetration, Response Surface Methodology.

1. Introduction

Topical antifungal therapies have gained considerable attention in recent years due to the rising incidence of fungal infections and the growing resistance to systemic antifungal agents [1]. Fungal infections can be difficult to treat due to the inherent challenges in drug delivery, including poor skin penetration, drug instability, and the complexity of targeting the fungal cells without damaging surrounding tissues [2].

Bifonazole, an imidazole antifungal agent, has demonstrated broad-spectrum antifungal activity, particularly against dermatophytes, yeasts, and molds [3]. However, its use is often limited by its low skin permeability and potential side effects from prolonged treatment.

In this context, novel drug delivery systems have been developed to enhance the efficacy of topical antifungal treatments [4].

One such system is invasomes, a promising vesicular drug delivery system. Invasomes are lipid-based nanocarriers that incorporate penetration enhancers to improve the transdermal delivery of drugs [5]. These nanocarriers can facilitate the deep penetration of antifungal agents like bifonazole into the stratum corneum, the epidermis, and even the dermis, which are essential for effective treatment of cutaneous fungal infections [6].

The incorporation of bifonazole into invasomes aims to overcome the limitations associated with conventional topical formulations. Invasomes not only enhance the solubility and stability of the drug but also improve its bioavailability and penetration, thereby ensuring better therapeutic outcomes [7].

The design of invasomes involves optimizing the composition of lipids and penetration enhancers to achieve a balance between skin permeation and drug release profile, ensuring that the antifungal agent is delivered effectively to the site of infection [8].

This approach has been increasingly studied in the development of formulations targeting skin infections, as it offers several advantages over traditional delivery systems, including increased bioavailability, sustained

release, and reduced systemic side effects [9].

The goal of this study is to design and develop invasomal formulations of bifonazole, evaluate their physicochemical properties, and assess their efficacy in treating topical fungal infections.

2. Materials and Methods

2.1. Material

For the development of invasomes containing bifonazole, a bifonazole gift sample was provided by Bayer Zydus Pharma Pvt. Ltd, Mumbai, India, serving as the active antifungal agent. Phosphatidylcholine from Himedia was used as the primary lipid component to form the vesicular structure of the invasomes, ensuring stability and efficient drug encapsulation. To enhance skin penetration, limonene, also sourced from Himedia, was incorporated as a natural terpene penetration enhancer, improving the drug's transdermal delivery. All other chemicals and solvents used in the study were of analytical grade, ensuring the purity and reliability of the formulations. These carefully selected materials were crucial in developing a stable and effective topical delivery system for bifonazole, aimed at improving the treatment of fungal skin infections.

2.2. Methods

A two factor two level Box Behnken design (BBD) was employed in optimization of Invasomes containing Bifonazole. The three variables (A – Phosphatidylcholine, B – Terpenes (limonene), C – Ethanol) were selected as independent variables [9]. These independent variables (factors) were selected at three different levels, i.e. low (-1), medium (0), and high (+1). The levels of factors and the obtained responses are shown in Table 1. The dependent variables (response) studied in this research work were Entrapment efficiency release (Y1, Entrapment efficiency, %) and Vesicle Size (Y2, nm). Seventeen runs of the experiment were evaluated for responses (Y1) and Y2 [9, 10, 11].

Table 1. Formulation variables and their levels in Box-Behnken experimental design

Formulation Variables			
Independent variables	**Level**		
	Low (-)	**Medium (0)**	**High (+)**
A: Phosphatidylcholine (% v/v)	0.25	0.5	0.75
B: Terpenes (% v/v)	0.1	0.15	0.2
C: Ethanol (ml)	5	7.5	10
Response variables			
R1: Entrapment efficiency			
R2: Vesicle Size			

2.2.1. Formulation of Bifonazole Loaded Invasomes

Bifonazole (10 mg) was loaded into invasomes by mechanical dispersion technique. Soya phosphatidylcholine (0.25 to 0.75% w/v) was added to ethanol and vortexed for 5 minutes. Drug and terpenes (0.1 to 0.2%) were added under constant vortexing, this mixture was sonicated for 5 minutes. Fine stream of phosphate buffer saline (up to 10% w/v) was added with syringe under constant vortexing. It was vortexed for additional 5 minutes to obtain final invasomal preparation (Table 2) [12, 13, 14, 15, and 16].

Table 2. Design matrix in Box-Behnken design for invasomes preparation

Std	Run	Factors					
		Coded Values			Actual Values		
		Factor A	Factor B	FactorC	Factor A : Phosphatidylcholine	Factor B: Terpenes	Factor C: Ethanol
3	1	-1	1	0	125	500	1
13	2	0	0	0	187.5	300	1
14	3	0	0	0	187.5	300	1
6	4	1	0	-1	250	300	0.5
2	5	1	-1	0	250	100	1
1	6	-1	-1	0	125	100	1
17	7	0	0	0	187.5	300	1
7	8	-1	0	1	125	300	1.5
4	9	1	1	0	250	500	1
12	10	0	1	1	187.5	500	1.5
15	11	0	0	0	187.5	300	1
10	12	0	1	-1	187.5	500	0.5
16	13	0	0	0	187.5	300	1
11	14	0	-1	1	187.5	100	1.5
9	15	0	-1	-1	187.5	100	0.5
8	16	1	0	1	250	300	1.5
5	17	-1	0	-1	125	300	0.5

Final Equation in Terms of Coded Factors

Entrapment efficiency = +66.61-0.4462A-1.32B-3.93C-1.18 AB-0.5475 AC+0.3000 BC+1.52 A^2-0.2493 B^2+1.14 C^2

Final equation in terms of actual factors

Entrapment efficiency = +89.74375-5.40700 Phosphatidylcholine + 32.51000 Terpenes - 4.23930 Ethanol - 94.00000 Phosphatidylcholine * Terpenes - 0.876000 Phosphatidylcholine* Ethanol + 24.29200 Phosphatidylcholine^2 - 99.70000 Terpenes^2 + 0.182920 Ethanol^2

Final Equation in Terms of Coded Factors

Vesicle Size = +183.44 - 0.5050 A + 14.09 B + 20.94 C + 14.01 AB - 10.55 AC - 1.81 BC - 1.11 A^2 + 0.5172 B^2 + 3.26 C^2

Final Equation in Terms of Actual Factors

Vesicle Size = +113.42250 - 25.91600 Phosphatidylcholine - 231.94500 Terpenes + 11.15910 Ethanol + 1120.80000 Phosphatidylcholine * Terpenes - 16.87200 Phosphatidylcholine * Ethanol - 17.68400 Phosphatidylcholine^2 + 206.90000 Terpenes^2 + 0.521960 Ethanol^2

2.2.2. Evaluation of prepared invasomes

2.2.2.1. Entrapment efficiency

Entrapment efficiency of bifonazole invasomes formulation was determined using centrifugation method [17, 18]. The entrapment efficiency of bifonazole in invasomes vesicle was determined by ultracentrifugation, 10 mL of invasomes formulation were collected in test tube. The amount of drug not entrapped in the invasomes was determined by centrifuging at 3,000 rpm and collecting the supernatant, the supernatant layer was separated, diluted with water suitably and drug concentration was determined at 260 nm using UV spectrophotometer.

2.2.2.2. Vesicle Size

The vesicle size and size distribution were determined using Dynamic Light Scattering (DLS) with a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). [19].

2.2.2.3. Morphology and Vesicular Shape

The morphology and shape of the invasome formulation 15 was analyzed using a transmission electron microscope (TEM) at an accelerating voltage of 100 kV. The samples were prepared by using negative staining with a 1% aqueous solution of phosphotungstic acid. A small amount of the invasomal formulation was placed on a carbon-coated grid and dried; the excess staining solution was carefully removed using whatman filter disks. Once the drying was done, the sample was examined under the microscope to determine its vesicular structure and morphology [20, 21].

2.2.3. Experimental data with predicted response

On the basis of experimental data with predicted response the formulation F15 was selected as optimized formulation for further evaluation.

2.2.4. In vitro skin permeation study

The in vitro skin permeation of invasomes system was studied using locally fabricated Franz's diffusion cell having an effective permeation area and receiver cell volume of 2 cm2 and 15 ml, respectively. The receptor cell contained 15 ml of phosphate buffer saline pH 7.4, which was constantly stirred with a magnetic stirrer at 100 rpm. Experiments were carried out for 24 h at 32 °C ± 1 °C. Samples were withdrawn through the receiver cell sampling port at 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, and 24.0 h and analyzed for drug content by UV spectrophotometer at 260 nm. The receptor cell after each withdrawal was replenished with an equal volume of fresh vehicle [22, 23, and 24].

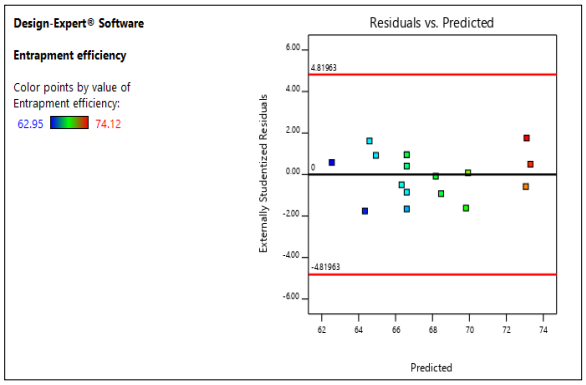
3. Results

The entrapment efficiency and vesicle size were predicted and optimized based on a combination of factors, including phosphatidylcholine (A), terpenes (B), and ethanol (C). The coded factor equations reveal that increasing phosphatidylcholine (A) and ethanol (C) tends to improve entrapment efficiency, while increasing terpenes (B) generally reduces it. The actual factor equation supports these findings, suggesting that high levels of phosphatidylcholine and terpenes enhance entrapment efficiency, while ethanol appears to negatively affect it. The interaction terms in the equations, such as the negative interaction between phosphatidylcholine and ethanol, indicate complex relationships between the formulation components that impact the final properties of the vesicular systems. Similarly, for vesicle size, increasing concentrations of phosphatidylcholine and terpenes lead to larger vesicle sizes, while ethanol appears to reduce the vesicle size. The positive interaction between phosphatidylcholine and terpenes also indicates that this combination is important in controlling vesicle size. The optimization of these parameters is crucial for developing formulations with high entrapment efficiency and controlled vesicle size, which are key factors in improving drug delivery performance. The transmission electron microscopy (TEM) analysis of formulation 15 revealed well-defined, spherical vesicles with a smooth surface morphology. The vesicle size observed through TEM was consistent with the size determined by dynamic light scattering (DLS), confirming the uniformity of the formulation. This correlation between the vesicle size and morphology supports the reliability of the DLS results and the successful optimization of formulation 15.

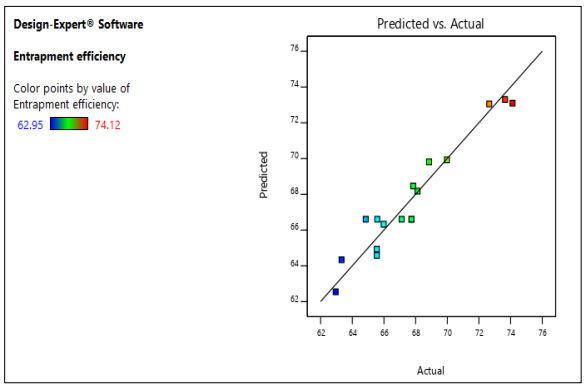
The experimental data in Table 3 for formulations F1 to F17 show variability in entrapment efficiency and vesicle size, which highlights the impact of different formulation factors. Formulation F15 stands out with an entrapment efficiency of 74.12% and a vesicle size of 154.45 nm, which was predicted accurately by the model, as seen in Table 4 (predicted vs. actual values). The close alignment between predicted and actual values, along with the random distribution of residuals in the graphical analysis (Figures 1a and 2a), indicates that the models for both entrapment efficiency and vesicle size are reliable and robust. The residuals vs. predicted and predicted vs. actual plots confirm that the model predictions were not biased and align well with experimental outcomes, further validating the optimization process.

Table 3. Results of entrapment efficiency and vesicle size of prepared formulations

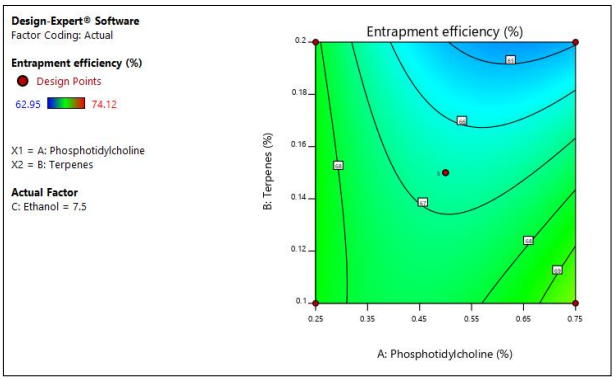
Formulation Code	Entrapment Efficiency (%)	Vesicle size (nm)
F1	68.85	180.32
F2	62.95	220.36
F3	67.74	185.65
F4	65.98	215.65
F5	65.55	210.36
F6	63.32	195.65
F7	67.74	183.32
F8	68.12	185.45
F9	65.55	197.74
F10	67.85	183.36
F11	65.58	184.45
F12	67.12	183.32
F13	64.85	180.45
F14	69.98	152.23
F15	74.12	154.45
F16	73.65	176.63
F17	72.65	150.45



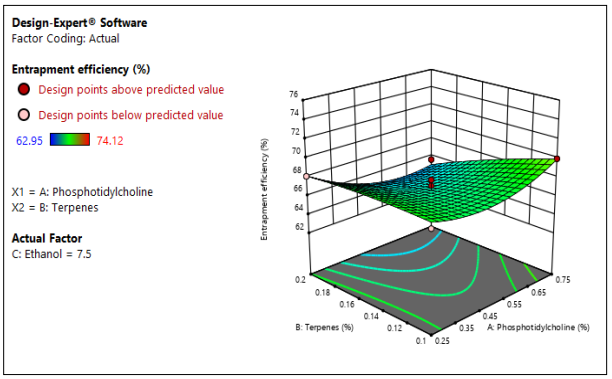
(a)



(b)



(c)



(d)

Fig 1. (a) Graph for entrapment efficiency Residuals vs. Predicted (b) Graph for entrapment efficiency Predicted vs. Actual (c) Contour graph for entrapment efficiency between Phosphatidylcholine vs. Terpenes (d) 3D surface graph for entrapment efficiency between Phosphatidylcholine vs. Terpene.

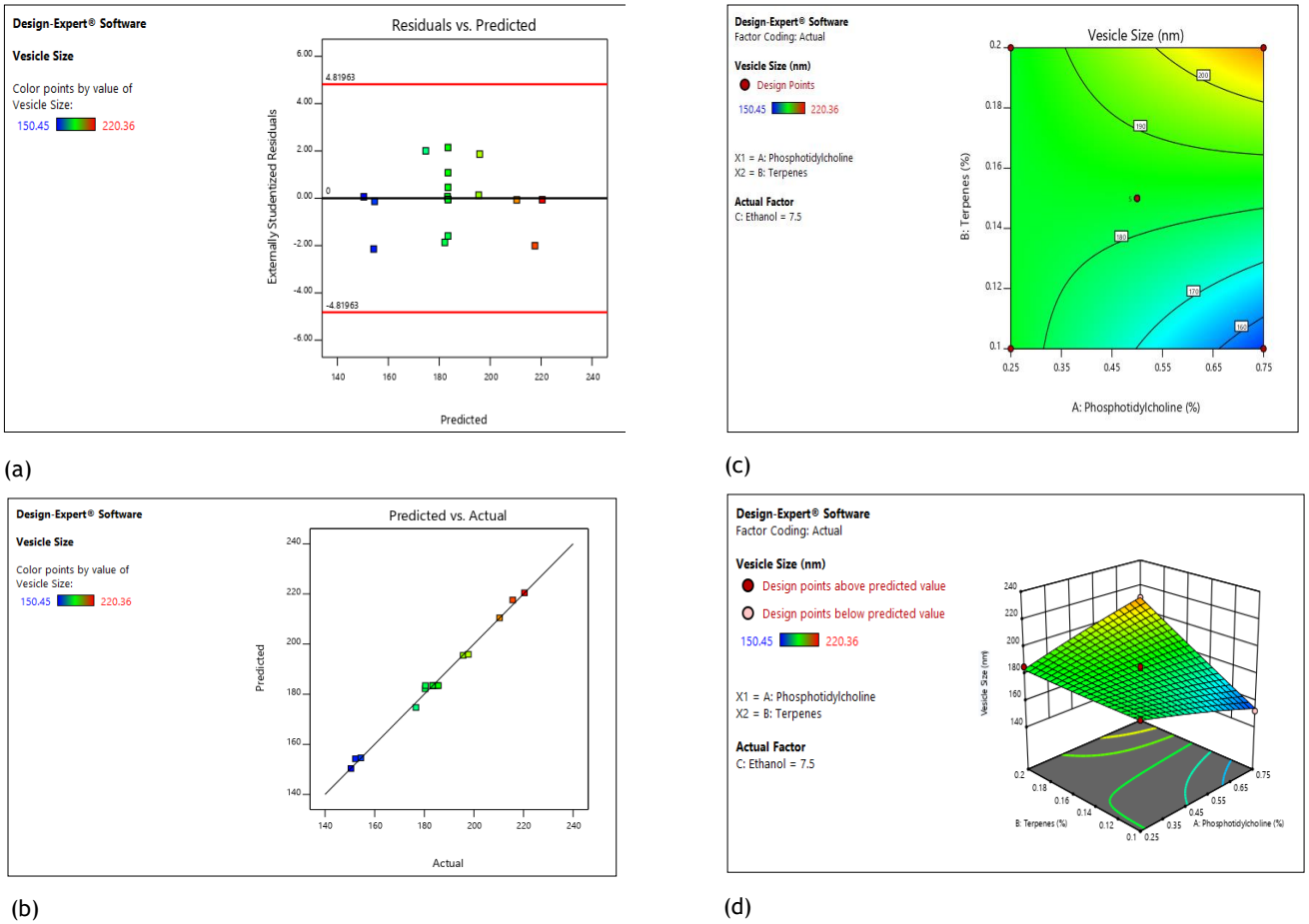


Fig. 2. (a) Graph for vesicle size Residuals vs. Predicted, (b) Graph for vesicle size Predicted vs. Actual (c) Contour graph for vesicle size Phosphatidylcholine vs. Terpenes (d) 3D surface graph for vesicle size Phosphotidylcholine vs. Terpenes.

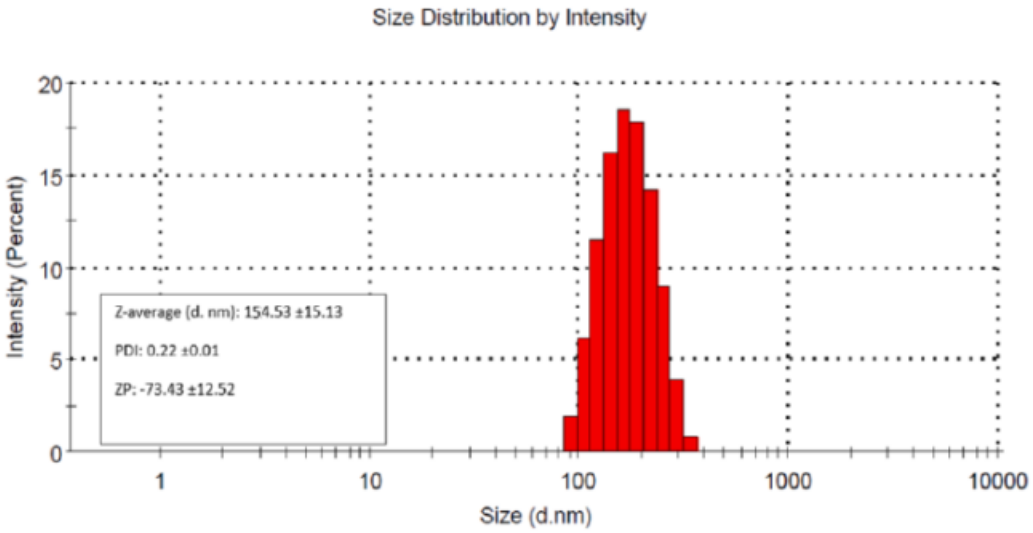


Fig. 3. Average particle size distribution of Formulation F15 obtained by DLS

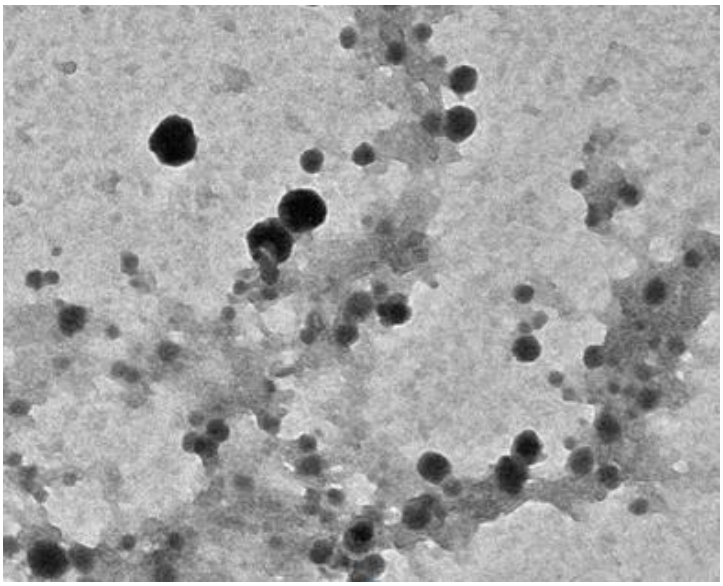


Fig. 4. Transmission electron microscope image of Formulation F15

Table 4. Experimental data with predicted response

Run Order	Formulation Code	Parameters	Actual Value	Predicted Value
15	F15	Vesicle size	154.45	154.61
		Entrapment Efficiency (%)	74.12	73.10
		Zeta potential	-39.48	

In terms of the in vitro skin permeation study, the results presented in Table 5 show that the invasomal formulation (F15) exhibits enhanced drug release compared to the plain drug. For instance, the plain drug release increased from 24.45% at 0.5 hours to 86.65% at 24 hours, while the invasomal formulation showed a more sustained release, with 14.65% at 0.5 hours and 62.23% at 12 hours. This suggests that the invasomes formulation is capable of controlling the release of the drug over an extended period, which could potentially improve therapeutic efficacy by maintaining steady drug levels in the target area, thus reducing the frequency of dosing and minimizing side effects.

Further analysis of the drug release kinetics in Table 6 provides insight into the release profile of formulation F15. The release data, when plotted against the square root of time (Fig. 7) and log time (Fig. 8), suggests that the release follows a zero-order kinetic model. This is typical of sustained-release systems, where the drug is released at a constant rate over time. The log cumulative percentage drug release increases steadily with time, and the log cumulative percent drug remaining decreases, indicating that the formulation is designed to provide sustained drug release. This is a promising feature for drug delivery systems, as it suggests that the invasomal formulation is effective in controlling drug release, potentially leading to improved patient compliance and more consistent therapeutic outcomes.

Table 5. Cumulative % drug release of from plain drug and invasomes formulation F15

Dissolution medium	Time (h)	% Cumulative Drug Release	
		Plain drug	Chitosan microspheres
Phosphate buffer saline pH 7.4	0.5	24.45	14.65
	1.0	36.65	26.65
	2.0	47.78	34.45
	4.0	55.65	41.12
	8.0	-	49.98
	12.0	-	62.23
	24.0	-	86.65

Table 6. *In vitro* drug release data for optimized formulation F15

Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
0.5	0.707	-0.301	14.65	1.166	85.35	1.931
1	1.000	0.000	26.65	1.426	73.35	1.865
2	1.414	0.301	34.45	1.537	65.55	1.817
4	2.000	0.602	41.12	1.614	58.88	1.770
8	2.828	0.903	49.98	1.699	50.02	1.699
12	3.464	1.079	62.23	1.794	37.77	1.577
24	4.899	1.380	86.65	1.938	13.35	1.125

* Average of three determinations

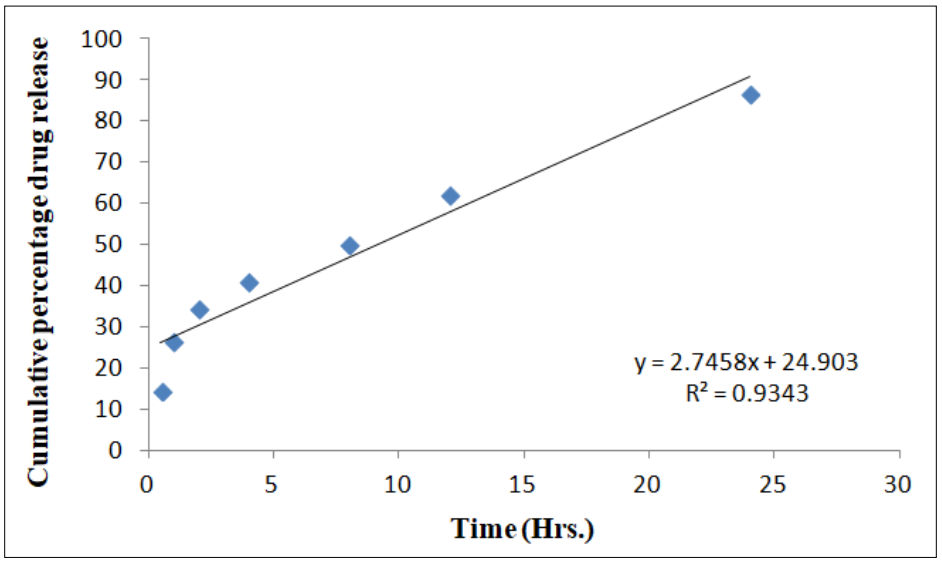


Fig. 5. Zero order release Kinetics (Cumulative % Drug Release vs Time (Hrs.)

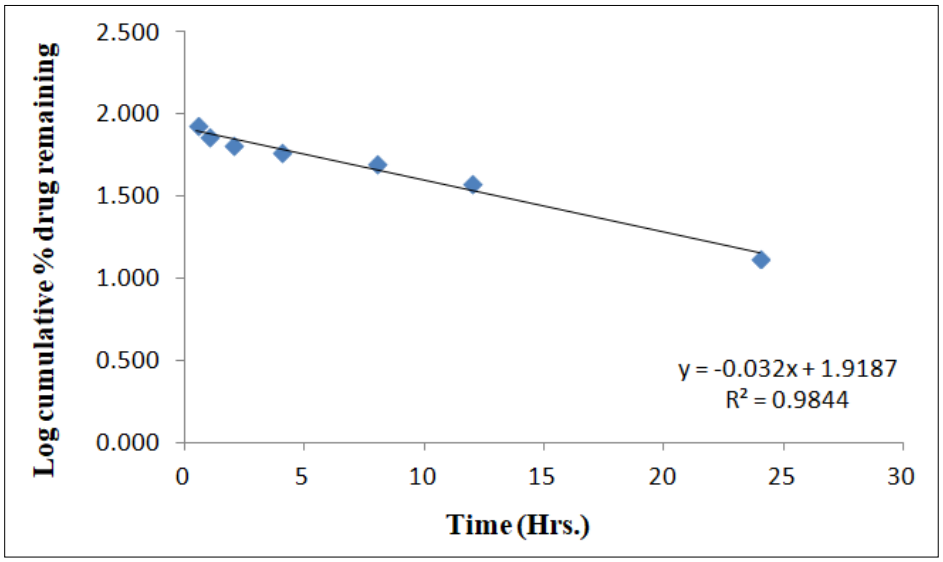


Fig. 6. First order release Kinetics (Log Cumulative % Drug remaining vs Time (Hrs.)

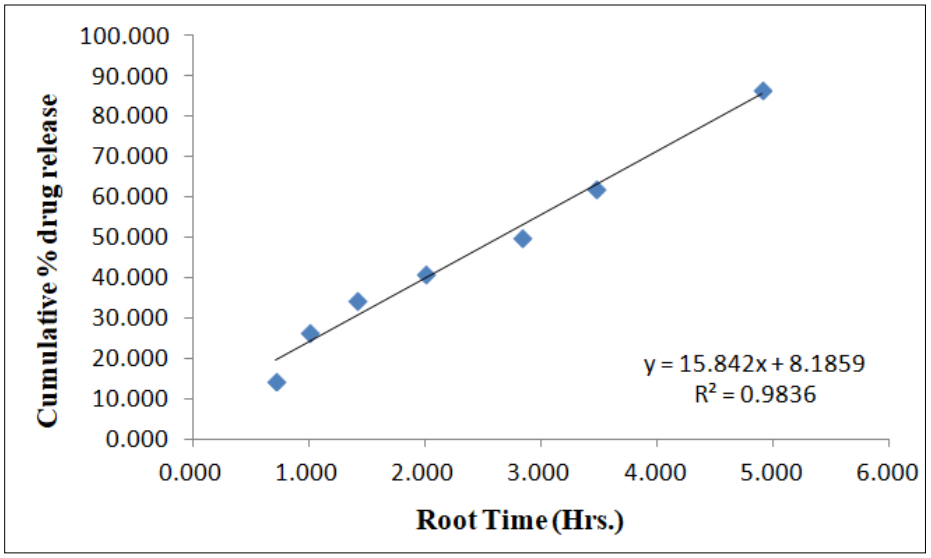


Fig 7. Higuchi release Kinetics (Cumulative % Drug release vs Root Time)

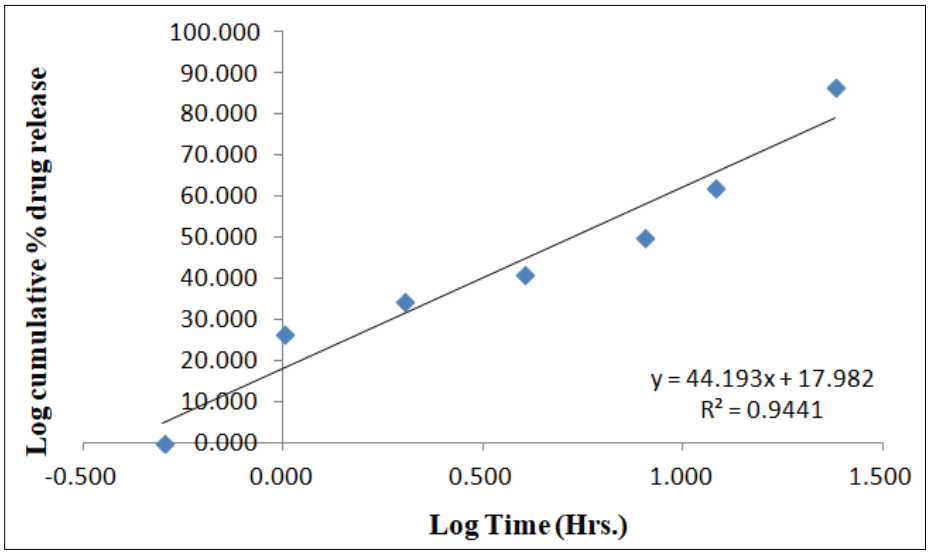


Fig 8. Korsmeyers Peppas release Kinetics (Log Cumulative % Drug release vs Log Time)

Table 7. Regression analysis data of optimized invasomal gel formulation

F. Code	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
	R^2	R^2	R^2	R^2
F15	0.9343	0.9844	0.9836	0.9441

4. Conclusions

In conclusion, the study demonstrates that invasomal formulations offer significant advantages in terms of entrapment efficiency, vesicle size, and drug release kinetics. By optimizing the formulation parameters using response surface methodology, it was possible to achieve formulations with desirable characteristics. The results of the in vitro skin permeation study suggest that the invasomal system can provide sustained drug release, which is beneficial for improving therapeutic efficacy and minimizing side effects. The drug release kinetics analysis further supports the potential of these formulations as controlled-release systems. These findings highlight the promise of invasomes in drug delivery applications, especially for treatments requiring sustained or localized delivery. Further investigations into the in vivo performance and long-term stability of these formulations will be valuable for confirming their clinical potential.

Author Contributions: Conceptualization, S.P. and S.B.; methodology, S.B.; validation, S.P., S.B.; investigation, S.P.; resources, S.B.; data curation, S.P.; writing—original draft preparation, S.P.; writing—review and editing, S.B.; visualization, S.B.; supervision, S.B.; project administration, S.B.; funding acquisition, S.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Acknowledgments: We are thankful to Principal, Madhav University, Abu Road Pindwara, Dist Sirohi - 307026, Rajasthan for supporting and guiding us.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kaur, I.P.; Kakkar, S. Topical delivery of antifungal agents. *Expert Opin. Drug Deliv.* **2010**, *7(11),* 1303–1327. DOI: 10.1517/17425247.2010.525230
2. Wu, S., Song, R., Liu, T. & Li, C. Antifungal therapy: Novel drug delivery strategies driven by new targets. *Adv. Drug Deliv. Rev.* **2023**, *199*, Art. No: 114967. DOI: 10.1016/j.addr.2023.114967
3. Rani, N.; Sharma, A.; Gupta, G.K.; Singh, R. Imidazoles as potential antifungal agents: A review. *Mini Rev. Med. Chem.* **2013**, *13*, 1626–1655. DOI: 10.2174/13895575113139990069
4. Mathur, M.; Devi, V.K. Potential of novel drug delivery systems in the management of topical candidiasis. *J. Drug Target* **2017**, *25*, 685–703. DOI: 10.1080/1061186X.2017.1331352
5. Jain, S.; Tripathi, S.; Tripathi, P.K. Invasomes: Potential vesicular systems for transdermal delivery of drug molecules. *J. Drug Deliv. Sci. Technol.* **2021**, *61*, Art. No: 102166. DOI: 10.1016/j.jddst.2020.102166.
6. Akhtar, N.; Verma, A.; Pathak, K.Topical delivery of drugs for the effective treatment of fungal infections of skin. *Curr. Pharm. Des.* **2015**, *21*, 2892–2913. DOI: 10.2174/1381612821666150428150456.
7. Nangare, S.; Dugam, S. Smart invasome synthesis, characterizations, pharmaceutical applications, and pharmacokinetic perspective: A review. *Futur. J. Pharm. Sci.* **2020**, *6*, Art. No :123. DOI: 10.1186/s43094-020-00145-8
8. Gaynanova, G., Vasileva, L., Kashapov, R., Kuznetsova, D., Kushnazarova, R., Tyryshkina, A., Vasilieva, E., Petrov, K., Zakharova, L. & Sinyashin, O. Self-assembling drug formulations with tunable permeability and biodegradability. *Molecules* **2021**, *26*, Art. No: 6786 DOI:10.3390/molecules26226786
9. Albash, R.; Al-Mahallawi, A.M.; Hassan, M.; Alaa-Eldin, A.A. Development and optimization of terpene-enriched vesicles (Terpesomes) for effective ocular delivery of fenticonazole nitrate: in vitro characterization and in vivo assessment. *Int. J. Nanomed.* **2021**, *16*, 609-621. DOI: 10.2147/IJN.S274290
10. Walbi, I.A.; Ahmad, M.Z.; Ahmad, J.; Algahtani, M.S., Alali, A.S.; Alsudi, S.A.; Aodah, A.H.; Albarqi, H.A. Development of a curcumin-loaded lecithin/chitosan nanoparticle utilizing a box-behnken design of experiment: formulation design and influence of process parameters. *Polymers* **2022**, *14,* Art. No: 3758. DOI: 10.3390/polym14183758
11. Alhalmi, A.; Amin, S.; Beg,.S.; Al-Salahi; R.; Mir,.S.R., Kohli, K. Formulation and optimization of naringin loaded nanostructured lipid carriers using Box-Behnken based design: In vitro and ex vivo evaluation. *J. Drug Deliv. Sci. Technol.* **2022**, *74*, Art. No: 103590. DOI: 10.1016/j.jddst.2022.103590
12. Sala, M.;. Elaissari, A.; Fessi, H. Advances in psoriasis physiopathology and treatments: Up to date of mechanistic insights and perspectives of novel therapies based on innovative skin drug delivery systems (ISDDS). *J. Control. Rel.* **2016**, *239*, 182–202. DOI: 10.1016/j.jconrel.2016.07.003
13. Dragicevic-Curic, N.; Gräfe, S.; Gitter, B.; Fahr, A. Efficacy of temoporfin-loaded invasomes in the photodynamic therapy in human epidermoid and colorectal tumour cell lines. *J. Photochem. Photobiol. B, Biol.* **2010**, *101*, 238–250. DOI: 10.1016/j.jphotobiol.2010.07.009
14. Sailaja, A.K.; Kirthi, R. Ethosomes and invasomes-A Vesicular Drug delivery carrier and their applications in transdermal drug delivery systems. *Pharm. Pharmacol. Res.* **2024**, *7*, 158. DOI: 10.31579/2688-7517/158
15. Raina, N., Rani R., Thakur V.K., Gupta M. New insights in topical drug delivery for skin disorders: from a nanotechnological perspective. *ACS omega* **2023**, *8*, 19145-19167. DOI: 1021/acsomega.2c08016
16. Samir,.B.; El-Kamel,.A.; Zahran,.N.; Heikal,.L. Resveratrol-loaded invasome gel: A promising nanoformulation for treatment of skin cancer. *Drug Deliv. Transl. Res.* **2024**, 1*4*, 3354-3370. DOI: 10.1007/s13346-024-01534-9
17. Teaima, M.H.; Eltabeeb, M.A.; El-Nabarawi, M.A., Abdellatif, M.M. Utilization of propranolol hydrochloride mucoadhesive invasomes as a locally acting contraceptive: In-vitro, ex-vivo, and in-vivo evaluation. *Drug Deliv.* **2022**, *29(1),* 2549–2560. DOI: 10.1080/10717544.2022.2100514
18. Badran, M.M.; Kuntsche, J.; Fahr, A. Skin penetration enhancement by a microneedle device (Dermaroller®) in vitro: Dependency on needle size and applied formulation. *Eur. J. Pharm. Sci.* **2009**, *36*, 511–523. DOI: 10.1016/j.ejps.2008.12.008,
19. Cunha, I.V; Campos, A.M.; Gerola, A.P.; Caon, T. Effect of invasome composition on membrane fluidity, vesicle stability and skin interactions. *Int. J. Pharm.* **2023**, *646*, Art. No: 123472. DOI: 10.1016/j.ijpharm.2023.123472
20. Jain,.S.; Tripathi,.S.;.Tripathi,.P.K., Invasomes: Potential vesicular systems for transdermal delivery of drug molecules. *J. Drug Deliv. Sci. Technol.* **2021**, *61*, Art. No: 102166. DOI: 10.1016/j.jddst.2020.102166
21. El-Kayal, M.; Hatem, S., A comparative study between nanostructured lipid carriers and invasomes for the topical delivery of luteolin: Design, optimization and pre-clinical investigations for psoriasis treatment. *J. Drug Deliv. Sci. Technol.* **2024**, *97*, Art. No: 105740. DOI: 10.1016/j.jddst.2024.105740
22. Salem, H.F.; Gamal, A.; Saeed, H.; Kamal, M.; Tulbah, A.S. Enhancing the bioavailability and efficacy of vismodegib for the control of skin cancer: in vitro and in vivo studies. *Pharmaceuticals* **2022**, *15(2),* Art. No: 126. DOI: 10.3390/ph15020126
23. Dragicevic-Curic, N.; Scheglmann, D.; Albrecht, V.; Fahr, A. Temoporfin-loaded invasomes: Development, characterization and in vitro skin penetration studies. *J. Control. Rel.* **2008**, *127*, 59–69. DOI: 10.1016/j.jconrel.2007.12.013
24. RefeKumar, B.; Sahoo, P.K.; Manchanda, S. Formulation, characterization and ex vivo study of curcumin nano-invasomal gel for enhanced transdermal delivery. *OpenNano* **2022**, *7*, Art. No: 100058. DOI: 10.1016/j.onano.2022.100058