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Original Research Article

FORMULATION AND EVALUATION OF CAPSAICIN LOADED LIPOSOMAL GEL FOR TREATMENT OF ALOPECIA

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ABSTRACT

In this study, Capsaicin loaded liposomal formulations were extensively evaluated for their potential as drug delivery systems, focusing on vesicle size, entrapment efficiency, *in-vitro* drug release, and stability. The vesicle size analysis revealed that formulations F1 to F6 ranged from 125.65 nm to 195.65 nm, with formulations F4 and F5 exhibiting sizes within the optimal range of 100-200 nm. Formulation F4, with a vesicle size of 125.65 nm, stood out for its superior entrapment efficiency of 74.65%, indicating efficient encapsulation of the drug compared to other formulations. The evaluation of liposomal gel formulations highlighted G2 as particularly promising due to its sustained drug release characteristics over a 10-hour period. The formulation exhibited an initial burst release within the first hour (46.65%) followed by a gradual release, achieving nearly complete drug release (98.85%) by the 10^{th} hour. This sustained release profile is advantageous for topical applications, ensuring prolonged therapeutic efficacy and potentially reducing dosing frequency. Regression analysis of formulation G2 confirmed its suitability for controlled drug release, with high coefficients of determination (R^2) for Zero Order (0.9539), First Order (0.8504), Higuchi's Model (0.9877), and Korsmeyer Peppas Equation (0.9877). Furthermore, stability studies conducted under controlled temperature conditions (4.0 ± 0.5°C and 28 ± 0.5ºC) for four weeks demonstrated no significant changes in physical appearance, average particle size, or drug content of the liposomal gel formulation. This stability profile indicates the formulation's robustness and potential for long-term storage without compromising its efficacy. In conclusion, the optimized liposomal gel formulation, particularly G2, shows promise as a viable platform for sustained drug delivery, offering controlled release characteristics and stability suitable for various pharmaceutical and biomedical applications.

Keywords: Liposomes, drug delivery, vesicle size, entrapment efficiency, sustained release, stability, controlled release kinetics, pharmaceutical formulations.

INTRODUCTION

Alopecia, a condition characterized by hair loss from the scalp or other areas of the body, presents a significant challenge in dermatology (Borda *et al.,* 2018). It encompasses various forms, including androgenetic alopecia (AGA), alopecia areata, and telogen effluvium, each with distinct etiologies and patterns of hair loss (Harries *et al.,* 2008; Otberg and Shapiro, 2012). Capsaicin, the active component found in chili peppers, has gained attention for its potential

therapeutic benefits in alopecia treatment (Siah *et al.,* 2021). Beyond its culinary use, capsaicin exhibits diverse pharmacological properties, including anti-inflammatory, antioxidant, and vasodilatory effects (Lee *et al.,* 2012). These properties are particularly relevant in alopecia treatment, where inflammation, oxidative stress, and impaired blood circulation contribute to hair follicle dysfunction and hair loss progression (Puri *et al.,* 2009).

Liposomal delivery systems offer a promising approach to enhance the efficacy of capsaicin in alopecia treatment (Bangham *et al.,* 1965; Torchilin, 2006). Liposomes are lipid-based vesicles composed of phospholipid bilayers that can encapsulate both hydrophilic and hydrophobic compounds, protecting them from degradation and facilitating controlled release (Allen & Cullis, 2013). By encapsulating capsaicin within liposomes, its stability can be improved, and its penetration into the hair follicles enhanced, thereby maximizing therapeutic efficacy while minimizing adverse effects on the skin (Fang *et al.,* 2004).

Numerous studies have explored the use of liposomal formulations to effectively deliver active compounds to the hair follicles, demonstrating their potential in enhancing drug permeation and retention in the targeted area (Van Zuuren *et al.,* 2016). For example, liposomal formulations have been successfully employed in dermatology for delivering drugs such as minoxidil and corticosteroids, showing improved therapeutic outcomes compared to conventional formulations. This research aims to explore the formulation and evaluation of capsaicin-loaded liposomal gels for the treatment of alopecia.

MATERIALS AND METHODS

Materials

For the development of liposomal gel formulation, a range of chemicals and excipients were utilized. These included capsaicin (procured from HI MEDIA), soya phosphatidyl choline (sourced from Ash Chemie India, Thane), and various chemicals obtained from S. D. Fine Chem. Ltd., Mumbai, such as disodium hydrogen phosphate, dipotassium hydrogen orthophosphate, sodium chloride, Carbopol 934P, methyl paraben, propyl paraben, and propylene glycol. Additionally, solvents including methanol, ethanol, and chloroform were purchased from Qualigens Fine Chemicals, Mumbai.

Methods

Formulation of Capsaicin loaded liposomes

Liposomes were prepared by rotator evaporation method given by Touitou *et al.,* (2000) with slight modification in which drug was dissolved in methanol to give a concentration of 1.0% w/v of drug solution. The accurately weighed amounts of phospholipids and surfactant were taken in a clean, dry, round-bottom flask and this lipid mixture was dissolved in minimum quantity of ethanol (5ml). The round bottom flask was rotated at 45º angle using rotator evaporator at 40°C in order to make uniform lipid layer. The organic solvent was removed by rotary evaporation under reduced pressure at the same temperature (40°C). Final traces of solvents were removed under vacuum overnight. The prepared lipid film in the inner wall of round bottom was hydrated with 2% w/v of drug solution in distilled water v/v,

followed by rotating the flask containing mixture of drug by rotation at speed of 60 rev/min for 1 hr. After complete hydration of film, the prepared formulation of liposomes was subjected to sonication at 4°C in 3 cycles of 10 minutes with 5 sec rest between the cycles. The prepared formulation was stored at 4°C in closed container till further use for analysis (Touitou *et al.,* 2000).

Table 1: Optimization of Capsaicin loaded liposomes

Formulation code	Soya PC (% w/v)	Span 80 (% w/v)	Drug (mg)	Ethanol (ml)
F1	4	2	50	5
F2	5	3	50	5
F3	6	4	50	5
F4	7	5	50	5
F5	8	6	50	5
F6	9	7	50	5

Characterization of liposomes

Vesicle size

Microscopic analysis was performed to determine the average size of prepared Liposomes. Formulation was diluted with distilled water and one drop was taken on a glass slide and covered with cover slip. The prepared slide was examined under trinocular microscopic at 400 X. The diameters of more than 150 vesicles were randomly measured using calibrated ocular and stage micrometer. The average diameter was calculated using the flowing formula (Touitou *et al.,* 2000).

$$Average\ Diameter = \frac{\Sigma n.d}{\Sigma\ n}$$

Where n = number of vesicles; d = diameter of the vesicles

Zeta potential

The size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the liposomes was based on the zeta potential that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9 % NaCl adjusted to a conductivity of 50 lS/cm (Hussain *et al.,* 2000).

Entrapment efficiency

Capsaicin entrapped within the liposomes was estimated after removing the unentrapped drug. The unentrapped drug was separated from the liposomes by subjecting the dispersion to centrifugation in a cooling centrifuge (Remi Equipments, Mumbai) at 18000 rpm at a temperature of 4°C for 45 minutes, where upon the pellets of liposomes and the supernatant containing free drug were obtained. The liposomes pellets were washed again with phosphate buffer to remove any unentrapped drug by centrifugation. The combined supernatant was analyzed for the drug content after suitable dilution with phosphate buffer solution by measuring absorbance at 282 nm using Labindia 3000+ spectrophotometer (Maurya *et al.,* 2010).

$$\%\ Entrapment\ Efficiency = \frac{Therotical\ drug\ content - Practical\ drug\ content}{\text{Therotical drug content}} \times 100$$

Preparation of gel base

Carbopol 934 (1-3%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and

then 10ml of propylene glycol was added to this solution. The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Gel was also prepared with plain drug by adding 10 mg of drug and dispersed properly by following same procedure given above. The same procedure was used to formulate liposome containing gel, liposomes preparation corresponding to 0.75% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base (Utreja *et al.,* 2011).

Characterization of liposomes containing gel

Measurement of viscosity

Viscosity measurements of prepared topical liposomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm (Nava *et al.,* 2011).

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted (Kamra *et al.,* 2017).

Drug content

Accurately weighed equivalent to 100 mg of topical liposome gel was taken in 10 ml volumetric flask, add 5 ml of methanol and sonicate it for 10 min and after sonication volume was made upto 10 ml with methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 0.1mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λ_{max} 282 nm (Leno *et al.,* 2012).

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

Spreadibility

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined by method reported by Multimer *et al.,* (1956). An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan.To determine spreadibility, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 6cm upon adding 20g of weight was noted, good spreadibility show lesser time to spread.

***In vitro* drug diffusion study**

The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion. The Franz diffusion cell has receptor compartment with an effective

volume approximately 60 mL and effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4.

The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32 ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength 282nm of drug.

RESULTS AND DISCUSSION

The evaluation and formulation of liposomes for the delivery of capsaicin in gel formulations are crucial steps in optimizing drug delivery systems for effective treatment of alopecia. Table 2 provides insights into the initial screening of liposome formulations based on vesicle size and entrapment efficiency. Notably, formulation F4 stood out with a vesicle size of 125.65 nm and a high entrapment efficiency of 74.65%, indicating its potential for optimal drug encapsulation and stability.

Moving to Table 3, which focuses on the optimized formulation, F4 maintained its desirable characteristics with consistent vesicle size and entrapment efficiency. The negative zeta potential (-38.78 mV) further underscores its stability, essential for sustained drug release and effective topical application.

Table 4 presents the results of liposomal gel formulations, where G2 exhibited high drug content (99.15%), suitable pH (7.01), and practical attributes such as spreadability (12.45 gm.cm/sec.) and viscosity (2485 cps). These parameters are critical for patient compliance and ensuring optimal skin absorption and retention of capsaicin.

The in-vitro drug release data in Table 5 for G2 demonstrate a sustained release profile over 10 hours, with cumulative drug release increasing steadily and fitting well with various kinetic models as shown in Table 6. The regression analysis indicates a good fit to Higuchi's model and Korsmeyer Peppas equation, suggesting controlled and predictable drug release kinetics from the liposomal gel formulation.

Table 2: Evaluations of liposomes for vesicle size and entrapment efficiency

Formulation	Vesicle Size (nm)	Entrapment efficiency (%)
F1	195.65±0.35	68.85±0.33
F2	179.98±0.25	71.12±0.45
F3	165.45±0.15	69.98±0.62
F4	125.65±0.36	74.65±0.41
F5	163.32±0.41	71.15±0.32
F6	186.65±0.32	70.32±0.15

Table 3: Vesicle size and entrapment efficiency of optimized formulation

Formulation Code	Vesicle Size (nm)	Entrapment Efficiency (%)	Zeta potential (mV)
F4	125.65±0.36	74.65±0.41	- 38.78

Table 4: Results of liposomes gel formulations

Code	Drug content (%)	pH	Spreadability (Gm.cm/sec.)	Viscosity (cps)
G1	98.95±0.15	6.85±0.05	14.85±0.22	2565±6
G2	99.15±0.32	7.01±0.03	12.45±0.32	2485±8
G3	97.57±0.25	6.92±0.04	9.65±0.18	23.85±3

Table 5: *In-vitro* drug release data for G2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	32.56	1.513	67.44	1.829
1	1.000	0.000	46.65	1.669	53.35	1.727
2	1.414	0.301	55.85	1.747	44.15	1.645
4	2.000	0.602	69.98	1.845	30.02	1.477
6	2.449	0.778	76.45	1.883	23.55	1.372
8	2.828	0.903	89.95	1.954	10.05	1.002
10	3.162	1.000	98.85	1.995	1.15	0.061

*Average of three reading

Table 6: Regression analysis data of liposomes gel formulation

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation
	R^2	R^2	R^2	R^2
G2	0.9539	0.8504	0.9877	0.9877

CONCLUSION

The study highlights the potential of liposomal formulations, particularly formulation G2, for delivering capsaicin effectively in the treatment of alopecia. The optimized vesicle size, high entrapment efficiency, favorable physicochemical properties of the gel, and controlled drug release kinetics support its further development and potential clinical application. Future research should focus on *in vivo* studies to validate these findings and assess the therapeutic efficacy and safety of this novel formulation in treating hair loss conditions.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Allen, T.M. & Cullis, P.R. (2013) Liposomal drug delivery systems: From concept to clinical applications. *Advanced Drug Delivery Reviews*, 65, 36–48.
- Bangham, A.D., Standish, M.M. & Watkins, J.C. (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. *Journal of Molecular Biology*, 13, 238–252.
- Borda, L.J., Perper, M. & Keri, J.E. (2018) Management of alopecia areata: Updates and algorithmic approach. *Journal of the American Academy of Dermatology*, 78, 15–24.
- Fang, J.Y., Fang, C.L., Liu, C.H. et al (2004) Enhancement of topical 5-aminolevulinic acid delivery by Er laser: Liposomal versus aqueous solutions. *Journal of Controlled Release*, 100, 65–77.
- Harries, M.J., Sinclair, R.D., Macdonald-Hull, S., Whiting, D.A., Griffiths, C.E. & Paus, R. (2008) Management of primary cicatricial alopecias: Options for treatment. *British Journal of Dermatology*, 159, 1–22.
- Ramzan, M., Ahsan, M.N., Ur Rehman, Z. & Ahmad, F.J. (2016) Elastic liposome-based gel for topical delivery of 5-fluorouracil: In vitro and in vivo investigation. *Drug Delivery*, 23, 1115–1129.
- Kamra, M., Diwan, A. & Sardana, S. (2017) Topical liposomal gel: A review. *International Journal of Pharmaceutical Sciences and Research*, 8, 2408–2414.
- Lee, S.H., Zheng, Z., Kang, J.S. et al (2012) Lipid-based drug delivery systems in oncology treatment. *Cancer Research and Treatment*, 44, 177–186.
- Leno Jenita, J.J., Madhusudhan, N.T. & Wilso, B. (2012) N, Manjula. D SBK. Formulation development and evaluation of. *World Journal of Pharmaceutical Research*, 1, 207–215.
- Multimer M. Spreadability determination by an apparatus (1956). *American Pharmacy, (Asso)*, 45, 212–218.
- Maurya, S.D., Prajapati, S.K., Gupta, A.K., Saxena, G.K. & Dhakar, R.C. (2010) Formulation development and evaluation of ethosome of stavudine.

Indian Journal of Pharmaceutical Education and Research, 44, 102–108.

- Nava, G., Piñón, E., Mendoza, L., Mendoza, N., Quintanar, D. & Ganem, A. (2011) Formulation and in vitro, ex vivo and in vivo evaluation of elastic liposomes for transdermal delivery of ketorolac tromethamine. *Pharmaceutics*, 3, 954–970.
- Otberg, N. & Shapiro, J. (2012) Hair growth disorders. In: *Fitzpatrick's Dermatology in General Medicine*, 8th edn (edited by L. A. Goldsmith, S. I. Katz, B. A. Gilchrest et al.). McGraw-Hill: New York, USA, pp. 981–992.
- Puri, A., Loomis, K., Smith, B., Lee, J.H., Yavlovich, A., Heldman, E. & Blumenthal, R. (2009) Lipid-based nanoparticles as pharmaceutical drug carriers: From concepts to clinic. *Critical Reviews in Therapeutic Drug Carrier Systems*, 26, 523–580.
- Siah, T.W., Chew, A.L., Fung, W.Y. et al (2021); 10 Recent advances in managing and understanding alopecia areata. *F1000 Faculty Rev*, 455, F1000Res.
- Torchilin, V.P. (2006) Multifunctional nanocarriers. *Advanced Drug Delivery Reviews*, 58, 1532–1555.
- Touitou, E., Dayan, N., Bergelson, L., Godin, B. & Eliaz, M. (2000) Ethosomes- Novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. *Journal of Controlled Release*, 65, 403–418.
- Utreja, P., Jain, S. & Tiwary, A.K. (2011) Localized delivery of paclitaxel using elastic liposomes: Formulation development and evaluation. *Drug Delivery*, 18, 367–376.
- Van Zuuren, E.J., Fedorowicz, Z., Carter, B., Andriolo, R.B. & Schoones, J. (2012) Interventions for female pattern hair loss. *Cochrane Database of Systematic Reviews*, 5, CD007628.