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FORMULATION AND CHARACTERIZATION OF CLINDAMYCIN LOADED INVASOMES FOR EFFECTIVE TREATMENT OF ACNE

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ABSTRACT

Acne vulgaris is a persistent, inflammatory skin condition that has become more and more burdensome for both sufferers and society. For that TDDS has significantly increases patient compliance and the therapeutic benefit as a whole. Invasomes are flexible nanoscale vesicular systems based on phospholipids that contain terpenes or a combination of terpenes, ethanol, and phosphatidylcholine, and have significantly greater percutaneous permeability. Thus, goal of this study is Formulation and Characterization of Clindamycin Loaded Invasomes for effective treatment of acne. The invasomal gel was formulated & evaluated as per the standard procedure. Results revealed that out of six formulation of invasome the F5 have highest entrapment efficiency of 73.32±0.14 % with vesicle size of 215.65±1.78nm. The zeta potential of F5 estimated to be -35.65. Further three formulations of invasomal gel were made of which highest drug content was observed in G-2 formulation which is 99.45±0.20. The viscosity & pH was observed to be 3312.85±0.35 cps & 6.81±0.15. Also, the extrudability and spreadibility observed to be 172±5±0.23g and 11.36±0.25 g.cm/sec. Additionally, the % Cumulative Drug Release for 12 hr noted to be 99.15 for G-2 formulation. The R^2 value was observed to be 0.992 which intended to follow higuchi release kinetics. The formulated invasomal gel observed to be stable for 3 months at 4.0 ±0. 2°C as s the drug content was observed to be 98.32 % also the physical appearance of gel seen to be normal. So, accordingly the formulated invasomal gel of clindamycin can be effectively used to treat acne.

Keywords: Invasome, Transdermal drug delivery system, Clindamycin, Invasomal gel, Acne vulgaris.

INTRODUCTION:

Acne vulgaris is a persistent, inflammatory skin condition that has become more and more burdensome for both sufferers and society. Acne, commonly referred to as acne vulgaris (AV), is a chronic skin condition that develops when the hair follicles become clogged with dead skin cells and skin oil. Blackheads or whiteheads, pimples, greasy skin, and potential scarring are its hallmarks. Depending on its severity, acne can leave skin scars in addition to causing emotional distress. Although acne can leave skin scars, it often has no long-term negative effects on health. The face, among other bodily components, is significant when it comes to one's perception of oneself. Even a small lesion in this area might make the patient feel uncomfortable

and huge. Low self-esteem, decreased social ties, and mental illnesses like anxiety and depression can all be brought on by this image (Bhate and Williams, 2013; Beylot *et al.*, 2014; Kraft and Freiman, 2011).

Thus, innovative drug delivery systems for currently available therapeutic compounds has become necessary recent years. In addition to enhancing the efficacy and safety of an existing drug, the invention of a novel delivery system also significantly increases patient compliance and the therapeutic benefit as a whole. Transdermal Drug Delivery System (TDDS) are discrete, self-contained dosage forms When placed to undamaged skin, invasome release the drug to the systemic circulation at a controlled pace through the skin. TDDS are dosage forms created to spread a therapeutically effective dose of medication throughout a patient's skin (Rastogi and Yadav, 2012; Brown *et al.*, 2006).

Invasomes are flexible nanoscale vesicular systems based on phospholipids that contain terpenes or a combination of terpenes, ethanol, and phosphatidylcholine, and have significantly greater percutaneous permeability than typical liposomes. Terpenes are strong permeation enhancers that quickly alter the stratum corneum's packing, dislodge its lipid structure, interact with intracellular proteins, and greatly increase stratum corneum drug partitioning. Invasomal vesicle penetration through the skin is facilitated by the synergistic effects of ethanol and terpenes present in the invasomes (Afreen and Shailaja, 2019; Nangare and Dugam, 2020).

A well -known drug for curing acne is clindamycin. Clindamycin is a semi-synthetic lincosamide antibiotic used topically to treat acne vulgaris as well as a number of serious infections caused by sensitive microorganisms. Its range of activity is rather limited and comprises gram-positive cocci and bacilli, gram-negative bacilli, and anaerobic bacteria. It's interesting to note that clindamycin has been used off-label to treat toxoplasmosis, malaria, and other protozoal infections (Guay, 2007; Smieja *et al*., 1998). Thus, goal of this study is Formulation and Characterization of Clindamycin Loaded Invasomes for effective treatment of acne.

MATERIALS AND METHODS

Chemical and reagents

Phosphotidylcholine, Terpenes, Ethanol obtained from Loba Chemical Pvt Ltd (Mumbai, India). Carbopol 934, Propylene glycolwere obtained from Merck Ltd, Mumbai, India. All solvents and reagents were of analytical grade.

Methods

Formulation and optimization of Clindamycin loaded Invasomes

Clindamycin (100mg) was loaded in to invasomes by mechanical dispersion technique. Soya Phosphatidylcholine (0.5 to 1.5% w/v) was added to ethanol and vortexed for 5 minutes (El-Nabarawi *et al.*, 2018). Drug (100mg) and terpenes (0.25%) were added under constant vortexing, this mixture was sonicated for 5 minutes. Fine stream of Phosphate buffer saline was added with syringe under constant vortexing. It was vortexed for additional 5 minutes to obtain final invasomal preparation.

Preparation of gel base

Carbopol 934 (1-3%w/v Invasome based gel formulation i.e. G-1 of 1%w/v, G-2 of 2%w/v, G-3 of 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 (Lakshmi *et al.*, 2014). 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. Final pH of the gel base was adjusted to 6.5. The same procedure was used to formulate Invasomes containing gel in which previously prepared Invasomes suspension was added. Invasomes preparation corresponding to 1% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

Evaluation of Invasomes

Entrapment efficiency

Entrapment efficiency of Clindamycin Invasomes formulation was determined using centrifugation method (Haag *et al.*, 2011). The entrapment efficiency of acyclovir in invasomes vesicle was determined by ultracentrifugation, 10mL of invasomes formulation were collect in test tube. The amount of drug not entrapped in the invasomes was determined by centrifuging at 3,000 rpm and collect the supernatant, the supernatant layer was separated, diluted with water suitably and drug concentration was determined at 300nm using UV spectrophotometer.

Vesicle Size

Microscopic analysis was performed to determine the average size of prepared invasomes (Ota *et al.*, 2003). 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.

Evaluation of Invasomes containing gel

Measurement of viscosity

A Brookfield viscometer (Brookfield Engineering Laboratories Inc. Rheo2,8, India) was used to evaluate the viscosity of invasomal gel at 37 ^{o}C.

pH: pH of individual and polyherbal gel formulation can be determined by using a pH meter.

Drug content

By dissolving 10 mg of the produced gel in a 100 ml volumetric flask and bringing the volume up to 50 ml with phosphate buffer pH 6.8, the drug content of the gel was determined. Whatman filter paper No. 41 was used to filter the content. A 25 ml volumetric flask was filled with 5 ml of the aforementioned solution. Using a Shimadzu UV/visible spectrophotometer, the amount of adapalene was measured at 230 nm against a blank.

Extrudability

Extrudability is an empirical test for determining the amount of force needed to remove a substance from a gel-filled tube. Collapsible tubes with invasomal gels within

were used to assess the formulation's extrudability. Pfizer's hardness tester evaluated extrudability. Aluminium tube was filled with 15g of gel, and the plunger was set to securely hold the tube in place. For 30 seconds, a pressure of 1 kg/cm2 was applied, and the mass of gel extruded was measured. This process is repeated three times along the tube's equidistant

Spreadability.

An excess of gel sample 1 gm was placed between two glass slides and a 150g weight was placed on slides for 5 minutes to compress the sample to a uniform thickness. The time required to separate the two slides was taken as a measure of spreadability.

***In-vitro* drug diffusion study**

By using dialysis tube diffusion techniques with a small modification, the in vitro drug release of entrapped drug from formulations was examined. The dispersions were created in control bags, which were then examined. In a dialysis bag is a gramme of medication. Threads were used to firmly enclose the dialysis bag. With the use of a glass rod, the dialysis bag was hung inside a beaker such that the formulation-containing area of the bag could dip into the buffer solution.A dialysis tube (MWCO 10000, Sigma) containing 1 ml of the sample was used to soak it in 20 ml of PBS (pH 6.8) for 12 hours before being knotted at both ends and placed in a different beaker. The beakers were put together above a magnetic stirrer so that there would be steady stirring at 100 rpm and a temperature of 32 1 °C. Intermittently, one ml of sample was removed from the receptor compartment and replaced with the same volume of solvent mixture.. The samples withdrawn and react with Methyl orange and extracted with 3 ml of chloroform are analysed spectrophotometrically at wavelength of 486nm.

Table 1: Formulation optimization of Clindamycin loaded Invasomes

Ingredient (%)	F1	F2	F3	F4	F5	F6
Clindamycin (mg)	100	100	100	100	100	100
Phosphotidylcholine (%)	1.0	1.5	2.0	1.0	1.5	2.0
Terpenes (%)	0.25	0.25	0.25	0.25	0.25	0.25
Ethanol (ml)	10	10	10	10	10	10

Table 2: Formulation optimization of gel base

Ingredient (%)	G-1	G-2	G-3
Drug (Invasomes equivalent to 0.1%)	1	1	1
Carbopol 934	1	2	3
Propylene glycol	0.2	0.2	0.2
Water (ml)	100	100	100

Table 3: Optimized formulation Invasomes

Formulation code- F5	
Clindamycin (mg)	100
Phosphotidylcholine (%)	1.5
Terpenes (%)	0.25
Ethanol (ml)	10

Table 4: Entrapment efficiency and average vesicle size

Formulation Code	% Entrapment efficiency	Average vesicle size (nm)
F1	68.98±0.15	274.45±2.15
F2	66.45±0.23	265.58±2.12
F3	67.98±0.14	244.65±1.15
F4	70.25±0.32	260.32±2.36
F5	73.32±0.14	215.65±1.78
F6	69.85±0.22	245.85±1.65

Table 5: Characterization of optimized formulation of invasomes

Formulation .	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F-5	215.65±1.78	73.32±0.14	-35.65

Table 6: Characterization of Invasomes gel

Gel formulation	Viscosity (cps)	pH	Drug Content (%)	Extrudability (g)	Spreadibility (g.cm/sec)
G-1	3545.65±0.25	6.74±0.22	97.85±0.15	168.85±0.15	12.25±0.32
G-2	3312.85±0.35	6.81±0.15	99.45±0.20	172±5±0.23	11.36±0.25
G-3	3145.58±0.32	6.32±0.32	98.12±0.14	165±2±0.14	10.25±0.15

Table 7: *In vitro* drug release study of optimized gel

S. No.	Time (hr)	% Cumulative Drug Release*		
		G-1	G-2	G-3
1	0.5	18.85	16.65	14.45
2	1	34.45	32.25	23.36
3	2	53.32	44.45	35.45
4	4	65.58	55.45	43.32
5	6	88.98	69.98	55.65
6	8	95.65	83.32	68.85
7	10	98.78	92.25	75.65
8	12	99.12	99.15	85.65

Table 8: *In vitro* drug release study of optimized gel formulation G-2

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	16.65
2	1	32.25
3	2	44.45
4	4	55.45
5	6	69.98
6	8	83.32
7	10	92.25
8	12	99.15

Table 9: *In-vitro* drug release data for optimized formulation G-2

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	16.65	1.222	83.31	1.222
1	1	0	32.25	1.523	66.68	1.523
2	1.414	0.301	44.45	1.659	54.42	1.659
4	2	0.602	55.45	1.819	34.13	1.819
6	2.449	0.778	69.98	1.859	27.68	1.859
8	2.828	0.903	83.32	1.933	14.35	1.933
10	3.162	1	92.25	1.954	10.02	1.954
12	3.464	1.079	99.15	1.995	1.15	1.995

Table 10: Regression analysis data of optimized gel formulation G-2

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
G-2	0.955	0.753	0.992	0.967

RESULTS AND DISCUSSION

The six different formulations of Invasome was formulated. It was seen that the formulation F5 have highest entrapment efficiency of 73.32±0.14 % with vesicle size of 215.65±1.78nm. The zeta potential of F5 estimated to be -35.65. Further three formulations of invasomal gel were made of which highest drug content was observed in G-2 formulation which is 99.45±0.20. The viscosity & pH was observed to be 3312.85±0.35 cps & 6.81±0.15. Also, the Extrudability and Spreadibility observed to be 172±5±0.23g and 11.36±0.25 g.cm/sec. Additionally, the % Cumulative Drug Release for 12 hr noted to be 99.15 for G-2 formulation. The R^2 value was observed to be 0.992 which intended to follow higuchi release kinetics. The formulated invasomal gel observed to be stable for 3 months at 4.0 ±0. 2°C. As the drug content was observed to be 98.32 % also the physical appearance of gel seen to be normal.

CONCLUSION

For greater bioavailability consideration, strong penetration capability of the invasome encapsulated agents via biological membrane, and their stability, invasomes have recently been examined by several researchers as a choice of topical or transdermal drug delivery system for curing acne. Clindamycin is the subject of the current formulation study, which aims to create an invasomal drug delivery system and assess its in vitro effectiveness. The terpene ratios used to produce the formulations varied. High entrapment efficiency is regarded as the ideal or best invasome formulation. Invasomal gel is a promising technique that could be utilized to deliver many other well-known medications used to treat various kinds of concurrent diseases transdermally.

DECLARATION OF INTEREST

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

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