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FORMULATION AND EVALUATION OF INVASOMES OF TAZAROTENE FOR POTENT ANTI ACNE EFFECT

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ABSTRACT

Tazarotene- loaded invasomes were prepared and evaluated for average vesicle size, zeta potential and entrapment efficiency. The effect of varying concentration of phosphatidylcholine on average vesicle size was studied. The Average vesicle size was found to be in the range of 220.14 ± 0.25 to 345.58 ± 0.41. Drug entrapment efficiency of Invasomes formulations is reduced with lowering concentration of lacithin. In general charged invasomes were more stable against aggregation and fusion than uncharged invasomes. The optimized formulation of invasomes was further incorporated into Carbopol gel base and evaluated for viscosity, pH, Spreadability and *in-vitro* drug release. The pH of the Gel was found to be in range of 6.75 ± 0.02 to 6.98 ± 0.02 which is good for skin pH. Drug content of tazarotene incorporated invasomes gel for formulation IG-1, IG-2 and IG-3 was found to be 96.65 ± 0.15, 99.12 ± 0.25 and 98.58 ± 0.32 respectively. In conclusion, the invasomes delivery systems may be a promising carrier for transdermal deliveryof tazarotene for the management of treatment of acne.

KEYWORD: Tazarotene, invasomes, transdermal, acne, lecithin.

INTRODUCTION

Acne is considered as one of the most widespread skin diseases.[1] When extreme disfiguration occurs it results in the development of severe consequences among theyoung people and may result in depression and suicide.[2] *Acne vulgaris* is the seconduppermost reason of suicide among skin diseases.[2] *Staphylococcus aureus* and *Propionibacterium acnes* have been attributed to acne vulgaris. Acne is an exclusive disease associated with skin occurs when sebaceous glands (SGs) attain special conditions. Hormonal changes,

such as puberty and menstrual cycles, seem to contribute to formation of acne vulgaris. An increase in somesex hormones, especially in androgens during puberty and pregnancy, cause the follicular glands to produce more sebum.[3] Transdermal delivery systems can avoid gastrointestinal drug absorption difficulties covered by gastrointestinal pH, enzymatic activity and drug interaction with food, drink and other orally administration drug.[4] They provided extended therapy with a single application, improving compliance over other dosage forms requiring more frequent dose administration e.g. Transdermal clonidine 7 day.[5]

Invasomes are novel and flexible vesicles containing a mixture of soy phosphatidylcholine (PC), terpenes, lyso PC, and ethanol with improved skin penetration.[6] Furthermore, invasomes have the same structural constituents as liposomes but contain terpene in their structure. It enhances permeation of drug through the skin for transdermal drug delivery.[7]

Tazarotene, is 6-[2-(4,4-dimethylthiochroman- 6-yl) ethynyl] ethyl nicotinate, a member of a new generation of receptor-selective synthetic retinoids, indicated in the mild to moderate plaque psoriasis disease, acne vulgaris, and photoaging. Dermal safety studies have specified that tazarotene did not demonstrate photoallergic or phototoxic potential. In recent times, the terpenes (single or ratio of terpenes), ethanol, etc. have been incorporated in a lipidic vesicular system (viz. invasomes) which enhanced the considerable penetration of vesicles (with active molecules) through the skin. Fascinatingly, invasomes are novel liposome-associated vesicles. Invasomas are bilayer vesicles, comprised of soya phosphatidylcholine (SPC), lysophosphatidylcholine (flexibility substances), terpenes, and ethanol (permeation enhancer).[8] Synergistic changes in all these components (i.e., unsaturated phospholipids, terpenes, and ethanol) are accomplished in deeper skin drug levelsthan in the case of traditional liposomes or a drug solution.[9]

The objective of this study was to develop and evaluate a novel invasomes based gel formulation containing tazarotene for targeted topical therapy of acne.

MATERIAL AND METHODS

Tazarotene was obtained from Bioplus Life Sciences Pvt. Ltd. Bangalore as gift. Phosphatidylcholines purchased from Thomas Baker, Mumbai, Carbopol 934p and Propylene Glycol from S. D. Fine Chem. Ltd., Mumbai. Instrurnts Labindia 3000+ UV -

Visible Spectrophotometer, Brook Field Viscometerm, Zeta Sizer Malvern Instruments, UK were used.

METHODS

Preformulation studies

Physical evaluation: It refers to the evaluation by sensory characters-taste, appearance, odor, feel of thedrug, etc.

Solubility: Solubility of the drug was determined by taking some quantity of drug (about 1-2 mg) in the test tube separately and added the 5 ml of the solvent (water, ethanol, methanol, 0.1N HCl, 0.1N NaOH, Chloroform and 7.4 pH phosphate buffer) Shake vigorously and kept for some time. Note the solubility ofthe drug in various solvents (at room temperature).

Melting point: It is one of the parameters to judge the purity of drugs. In case of pure chemicals, melting points are very sharp and constant. A small quantity of powder was placed into a fusion tube. That tube was placed in the melting point determining apparatus (Chemline) containing castor oil. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

FTIR Spectroscopy: Infra- red spectrum is an important record which gives sufficient information about the structure of a compound. This technique provides a spectrum containing a large number of absorption band from which a wealth of information can be derived about the structure of an organic compound.

Loss on drying: Loss on drying is directly measured by IR moisture balance. Firstly calibrated the instrument by knob then taken 5.000 gm sample (powder) and set the temp at 100°C to 105°C for 15 minutes and constant reading set the knob and check % moisture.

Moisture content determination: Karl Fischer volumetry is used for samples with high water content, *i.e.* 1- 100 mg per sample. An iodine-containing solution serves as titrating agent. The water content of the sample is calculated using titration volume and titer of the titrating agent. One-component reagents conveniently contain all reactants (Iodine, sulfur dioxide and a base) dissolved in a suitable alcohol in one solution, whereas two-component reagents contain all necessary reactants separated in two different solutions to

enhance the rapidity of the Karl Fischer reaction and the titer stability of the titrating agent.

Determination of λ_{max} of Tazarotene: The λ_{max} of Tazarotene was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer. Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.4 pH phosphate buffer solution in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.4 pH phosphate buffer solution prepare suitable dilution to make it to a concentration range of 10-50 µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). The spectrum peak point graph of absorbance of Tazarotene versus wave length was shown in figure.

Formulation Optimization of Tazarotene loaded Invasomes

Tazarotene was loaded in to invasomes by mechanical dispersion technique. Soya Phosphatidylcholine (0.5 to 1% w/v) was added to ethanol and vortexed for 5 minutes [75-76]. Drug and terpenes (0.5 to 1.5%) were added under constant vortexing, this mixture was sonicated for 5 minutes. Fine stream of Phosphate buffer saline (upto 10% w/v) was added with syringe under constant vortexing. It was vortexed for additional 5 minutes to obtain final invasomal preparation.

Evaluation of Invasomes

Entrapment efficiency: Entrapment efficiency of Tazarotene Invasomes formulation was determined using centrifugation method. 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 351 nm using UV spectrophotometer.

$$\% \text{ Entrapment efficiency} = \frac{(\text{Drug added} - \text{free or unntraped drug})}{\text{Drug added}} \text{ X } 100$$

Vesicle Size: Microscopic analysis was performed to determine the average size of prepared invasomes.[79] 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.

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

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

Preparation of Gel Base

Carbopol 934 (1-3% w/v -Invasome based gel formulation i.e. IG-1 of 1% w/v, IG 2 of 2% w/v, IG-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.[77] 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 0.1% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

Evaluation of Invasomes containing gel

Measurement of viscosity: Viscosity measurements of prepared topical Invasomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm.

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.

Drug content: Accurately weighed equivalent to 100 mg of topical Invasomes gel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL 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} 351 nm.

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. 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 topplate to cover a distance of 10 cm upon adding 80 g of weight was noted. Good spreadibility show lesser time to spread.

$$Spreadibility\ (g.cm/sec) = \frac{Weight\ tide to Upper\ Slide \times Lenth\ moved\ onthe\ glass\ slide}{Timetakento\ slide}$$

***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 3.14 sq.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 thermostatichot 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 of 351 nm.

Stability Studies

Stability study was carried out for drug loaded invasomes at two different temperatures i.e. refrigeration temperature (4.0 ± 0.2°C) and at room temperature (25-28 ± 2°C) for 3 weeks. The formulation subjected for stability study was storedin borosilicate container to avoid

any interaction between the formulation and glass of container. The formulations were analyzed for any physical changes and drugcontent.

RESULTS AND DISCUSSION

Preformulation studies

Physiochemical Properties of Tazarotene: the drug Tazarotene was a light yellow tasteless and odorless powder insoluble in water soluble in 0.1 N NaOH and 0.1 N HCl and freely soluble in ethanol and methanol, having melting point 95-96^{o}C. FT-IR studies were suggested that pure drug and excipients are compatible, loss on drying was found 0.156±0.002% and moisture content was 0.1094 by Karl Fischer titration. Wavelength maxima (λ_{max}) of tazarotene in phosphate buffer pH 7.4 at 351nm. Calibration curve of tazarotene in phosphate buffer pH 7.4 was obtained with r^2 =0.998.

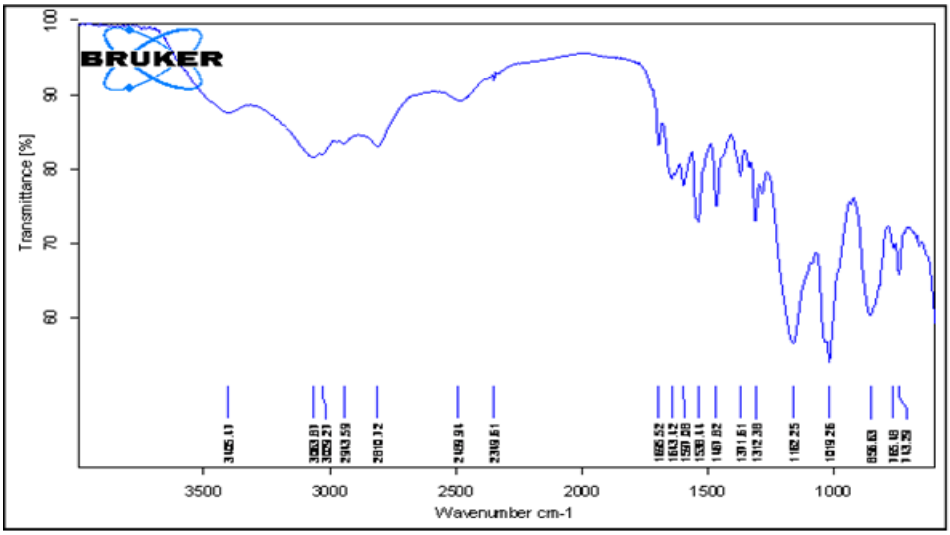

Figure 1: FT-IR Spectrum of Pure Drug (Tazarotene).

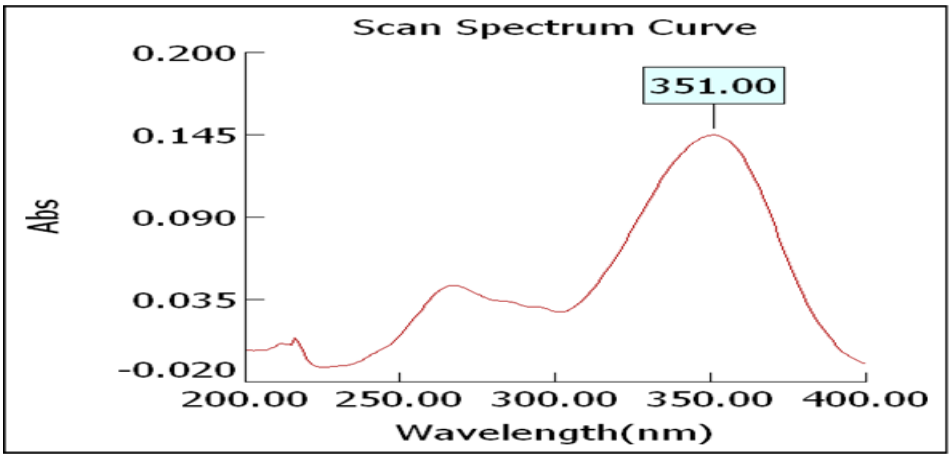

Figure 2: Wavelength maxima of tazarotene in phosphate buffer pH 7.2.

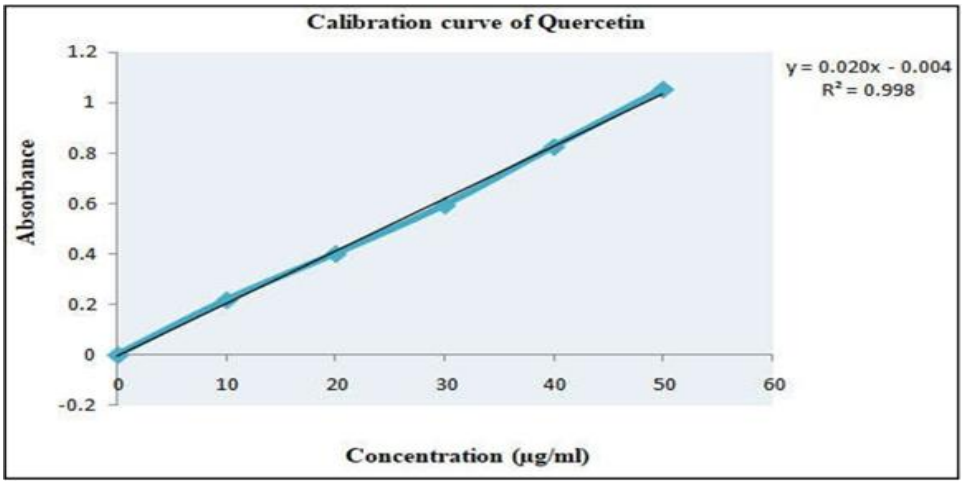

Figure 3: Calibration curve of tazarotene in phosphate buffer pH 7.4.

Preparation of Tazarotene loaded Invasomes

Table 1: Formulation optimization of Tazaroteneloaded Invasomes.

Ingredient (%)	F1	F2	F3	F4	F5	F6
Tazarotene (mg)	50	50	50	50	50	50
Phosphotidylcholine (%)	0.5	0.5	0.5	1	1	1
Terpenes (%)	0.25	0.25	0.25	0.5	0.5	0.5
Ethanol (ml)	5	5	5	5	5	5

Characterization of Tazarotene loaded Invasomes

Table 2: Entrapment efficiency and average vesicle size.

Formulation Code	% Entrapment efficiency	Average vesicle size (nm)
F1	65.58 ± 0.25	256.65 ± 0.25
F2	68.54 ± 0.36	312.56 ± 0.32
F3	63.32 ± 0.14	285.45 ± 0.45
F4	65.56 ± 0.35	345.58 ± 0.41
F5	76.65 ± 0.24	220.14 ± 0.25
F6	69.95 ± 0.18	256.65 ± 0.54

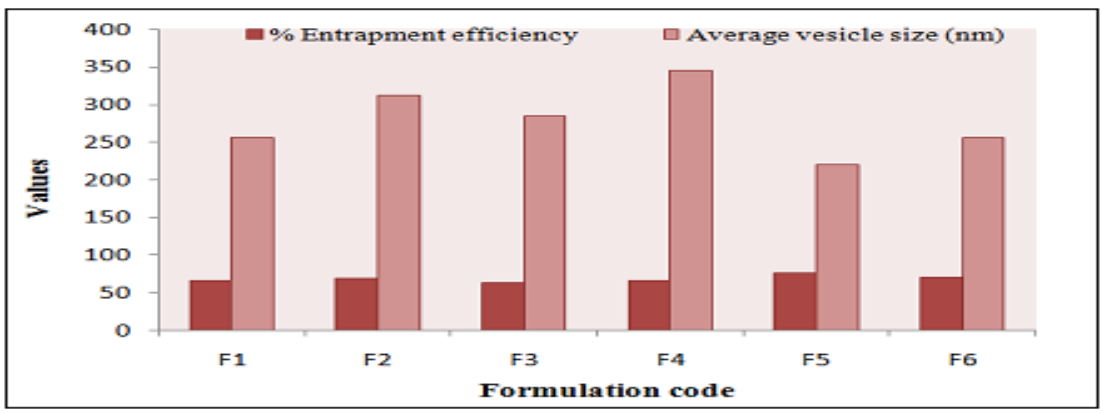

Figure 4: Graph of % entrapment efficiency and average vesicle size of all formulation F1 to F6.

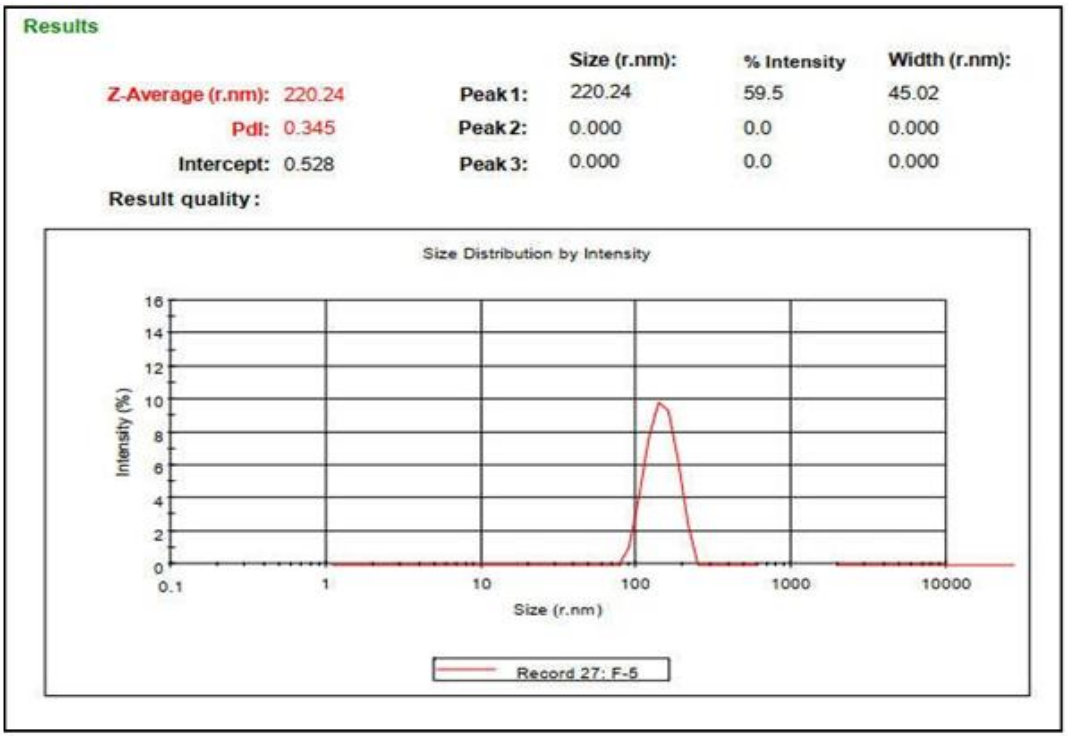

Figure 5: Graph of average vesicle size (nm) of optimized formulation F-5.

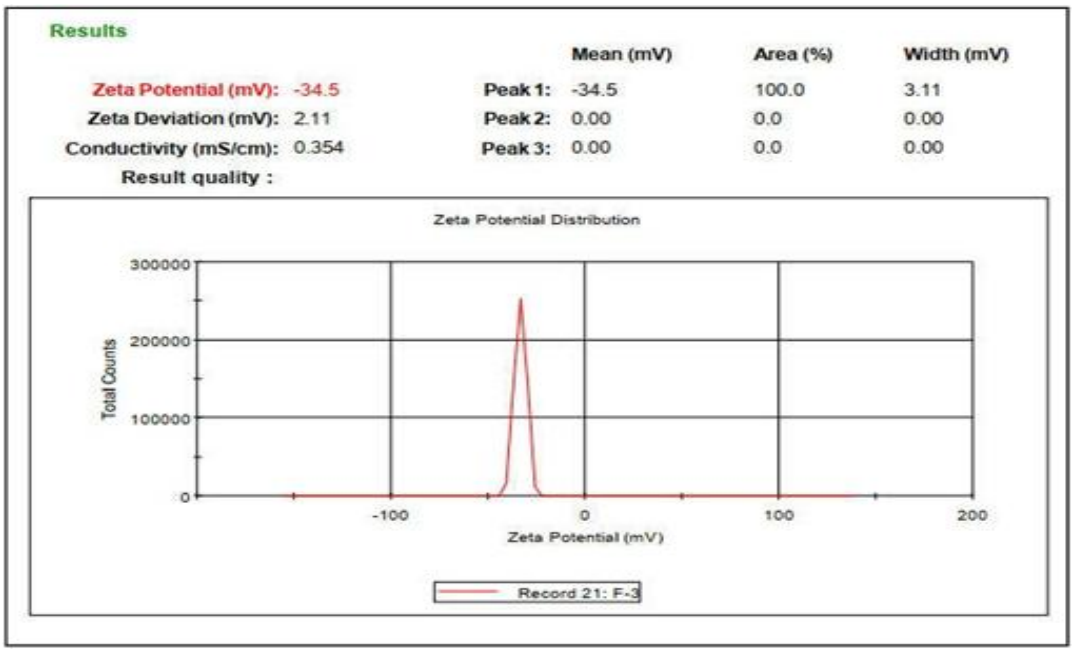

Figure 6: Graph of zeta Potential (mV) optimized formulation F-5.

Table 3: Characterization of gel based formulation of Invasomes.

Gel Formulation	Viscosity (cps)	pH	Drug Content (%)	Extrudability (g)	Spreadibility (g.cm/sec)
IG-1	3565±15	6.75±0.02	96.65±0.15	145.5±0.5	11.25±0.25
IG-2	3325±12	6.82±0.03	99.12±0.25	165.6±0.4	10.23±0.32
IG-3	3045±14	6.98±0.02	98.58±0.32	163.1±0.2	9.85±0.14

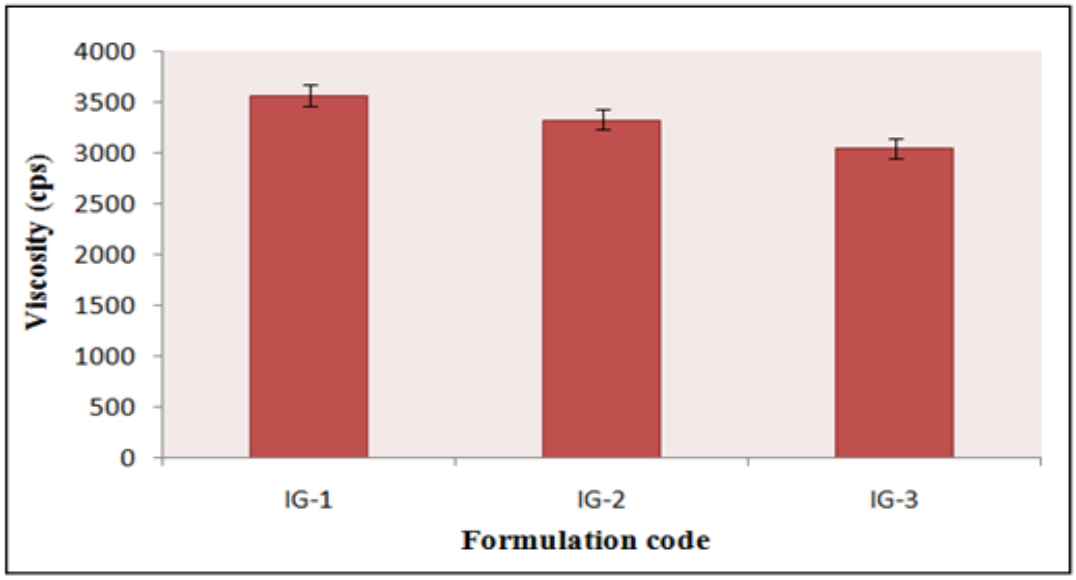

Figure 7: Result of viscosity (cps) of formulation IG1 to IG3.

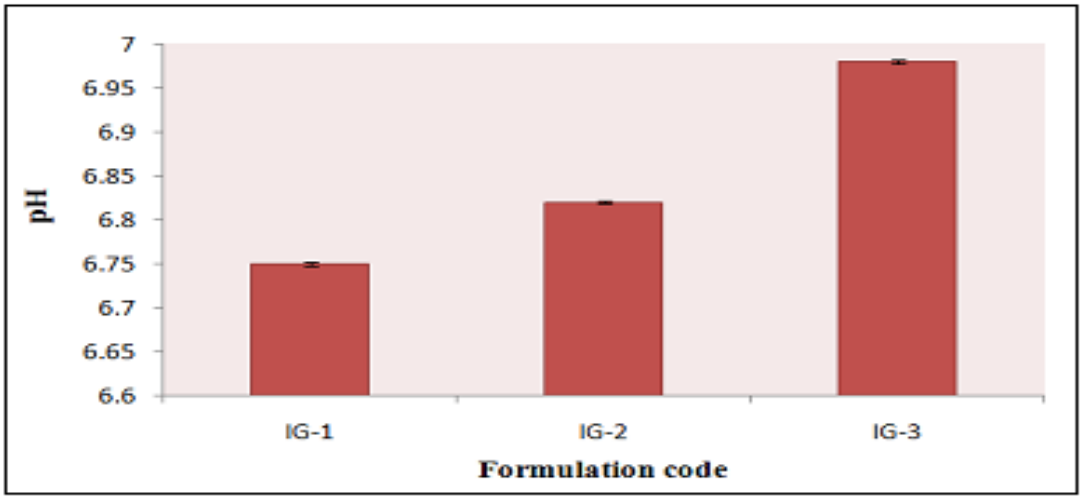

Figure 8: Result of pH of formulation IG1 to IG3.

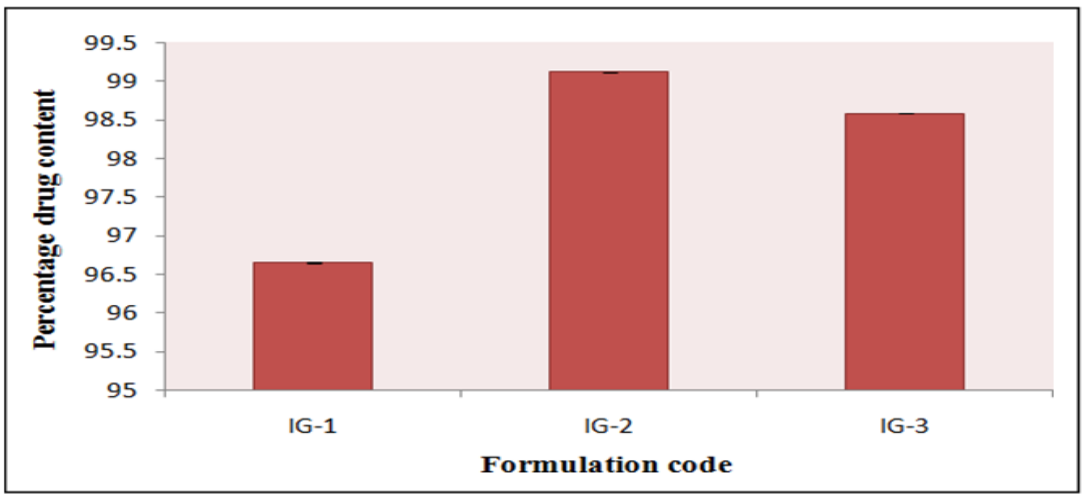

Figure 9: Result of percentage drug content of formulation IG1 to IG3.

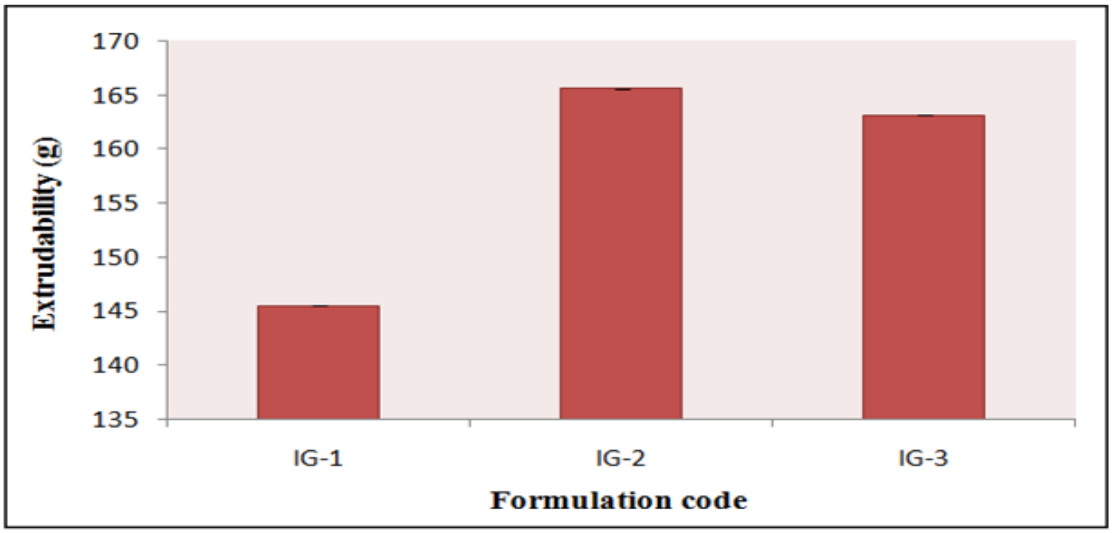

Figure 10: Result of percentage extrudability of formulation IG1 to IG3.

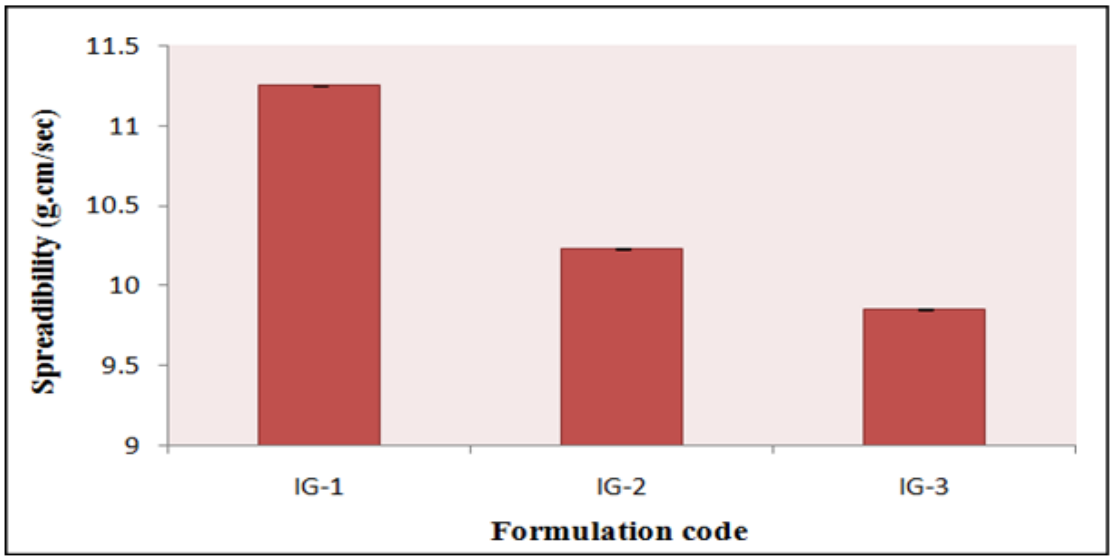

Figure 11: Percentage spreadibility (g.cm/sec) of formulation IG1 to IG3.

Table 4: In vitro drug release study of prepared optimized gel formulation IG-2.

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	11.25±0.25
2	1	23.36±0.32
3	2	36.65±0.45
4	4	43.32±0.65
5	6	64.74±0.21
6	8	79.98±0.14
7	10	89.95±0.25
8	12	98.85±0.33

Release kinetics of Invasomes encapsulated formulation IG-2.

Table 5: *In-vitro* drug release data for optimized formulation IG-2.

Time (h)	Square Root of Time(h)	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	- 0.301	11.25	1.051	88.75	1.948
1	1	0	23.36	1.368	76.64	1.884
2	1.414	0.301	36.65	1.564	63.35	1.802
4	2	0.602	43.32	1.637	56.68	1.753
6	2.449	0.778	64.74	1.811	35.26	1.547
8	2.828	0.903	79.98	1.903	20.02	1.301
10	3.162	1	89.95	1.954	10.05	1.002
12	3.464	1.079	98.85	1.995	1.15	0.061

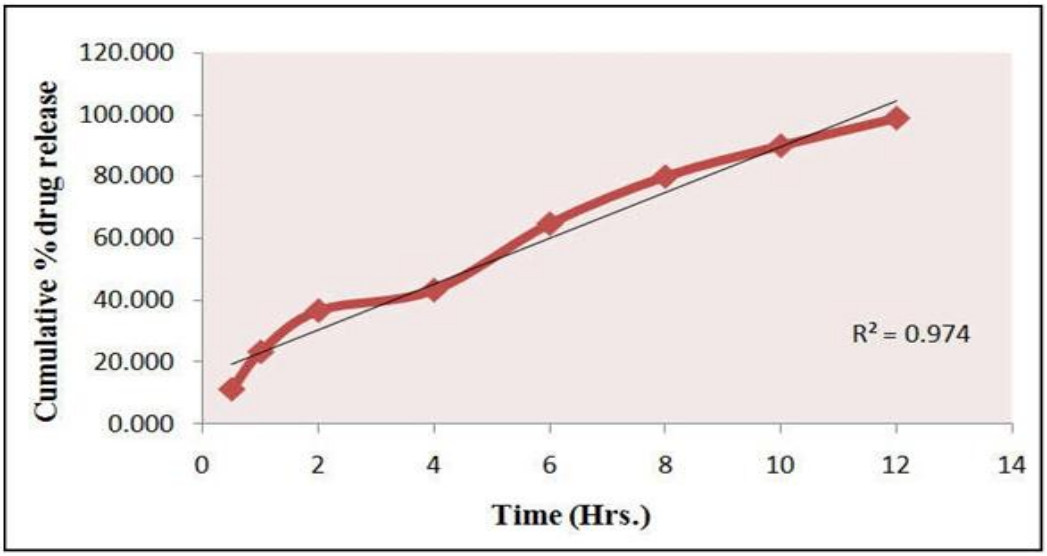

Figure 12: Cumulative % drug released Vs Time (Zero Order Kinetics).

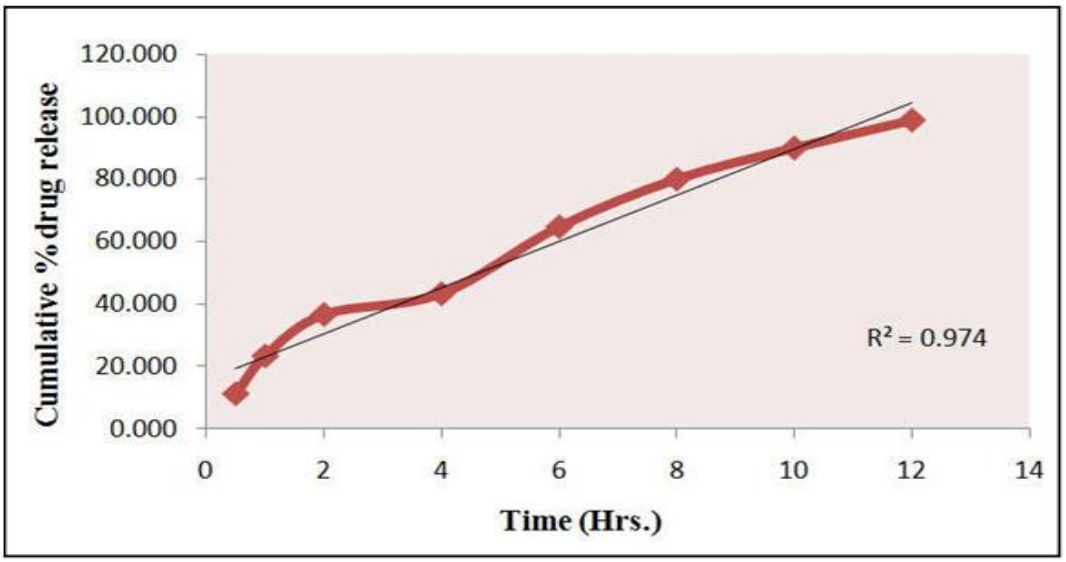

Figure 13: Log cumulative % drug remaining Vs Time (First Order Kinetics).

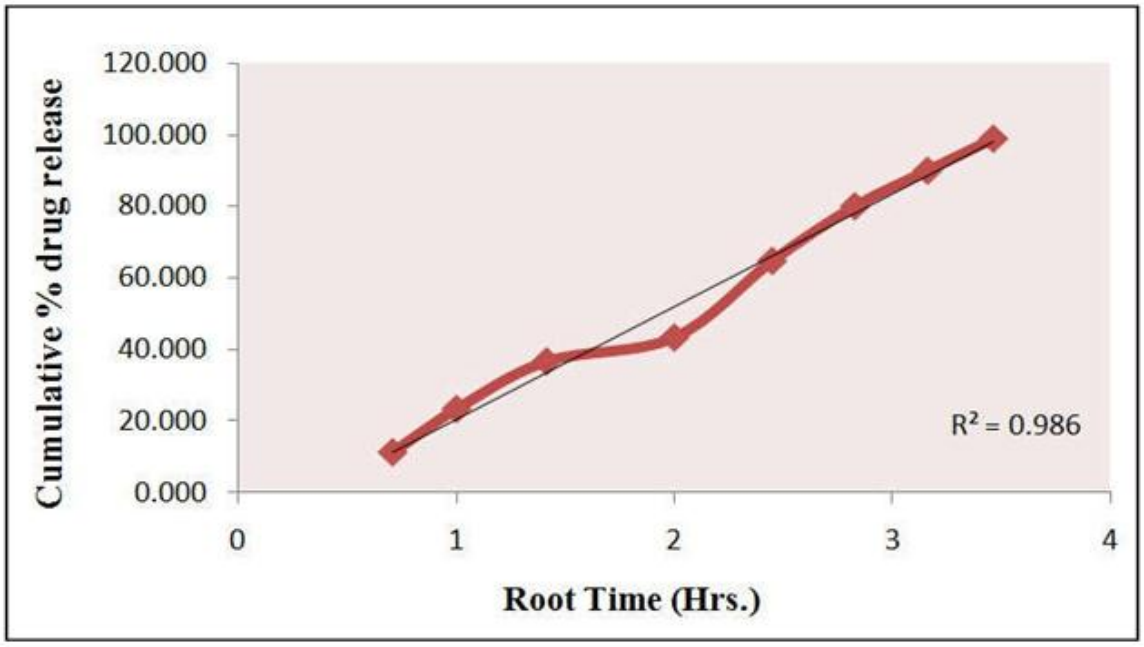

Figure 14: Cumulative % drug release Vs Root time (Higuchi Release Kinetics).

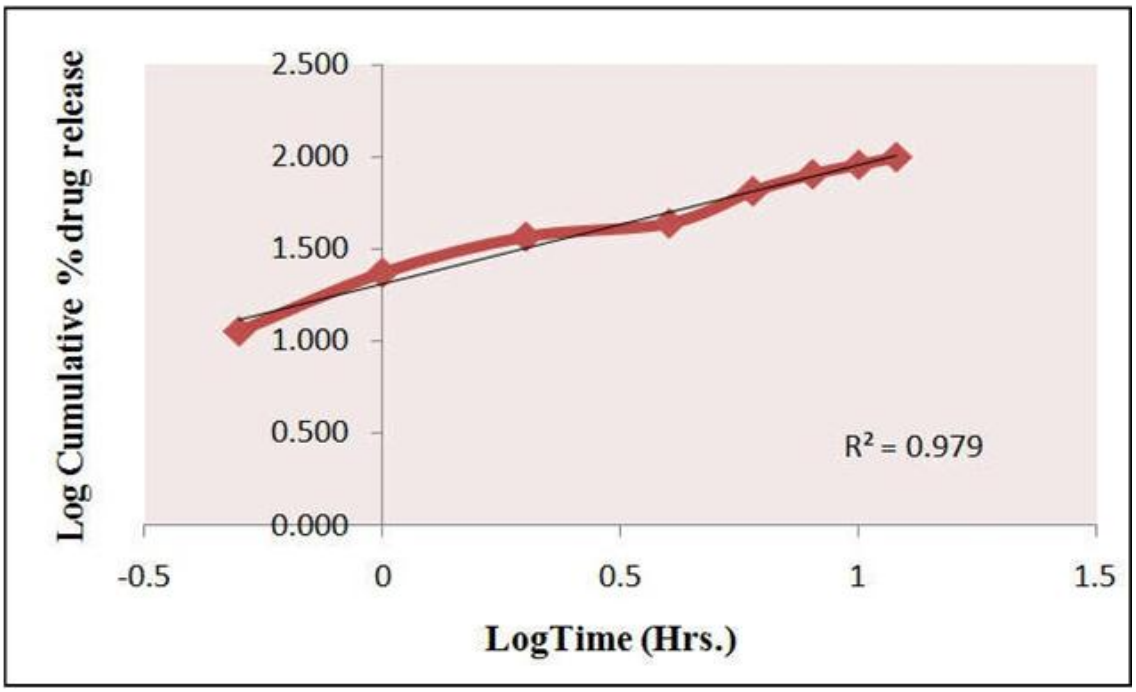

Figure 15: Log Cumulative % drug release Vs Log time (Korsmeyer Peppas Model).

Table 6: Regression analysis data of optimized gel formulation IG-2.

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
IG-2	0.974	0.850	0.986	0.979

Results of Stability

Table 7: Stability of optimized formulation of invasomes.

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temp.	4.0 ± 0. 2°C	25-28 ± 2°C	4.0 ± 0. 2°C	25-28 ± 2°C	4.0 ± 0. 2°C	25-28 ± 2°C
Average particle size (nm)	220.10±0.12	265.58±0.32	225.45±0.25	347.85±0.32	228.98±0.14	380.25±0.14
% E E	76.45±0.32	65.58±0.15	75.65±0.25	62.45±0.65	73.36±0.45	55.45±0.37
Physical Appearance	Normal	Turbid	Normal	High turbid	Normal	High turbid and agglomeration

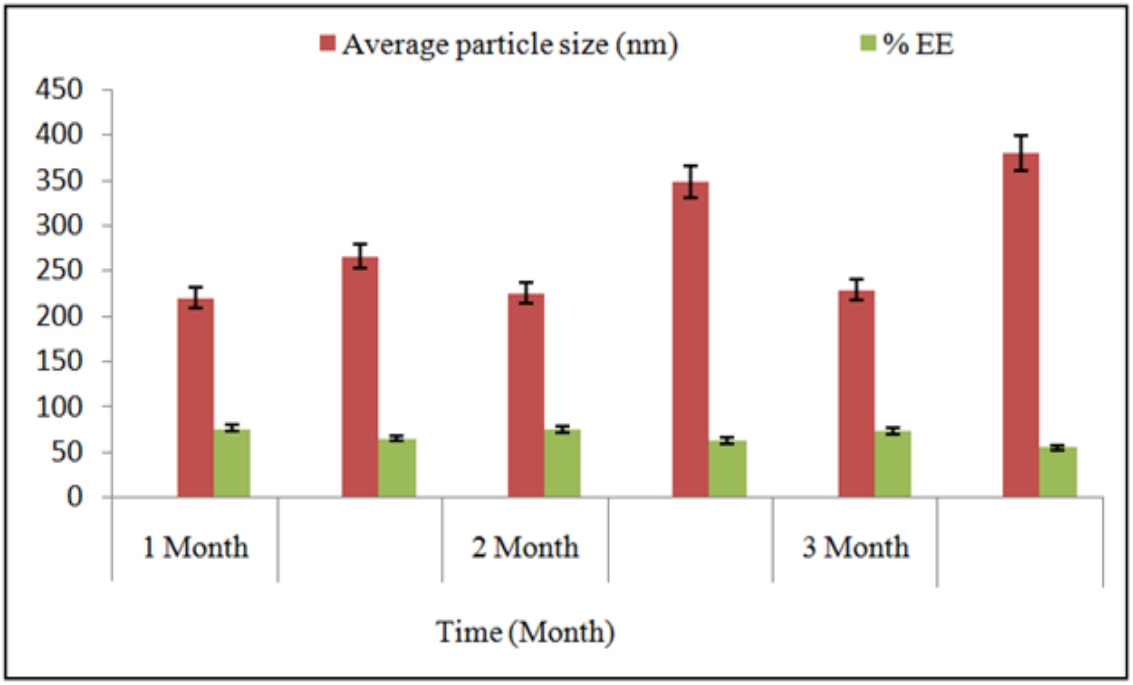

Figure 16: Graph of stability study of optimized formulation of Invasomes.

DISCUSSION

The effect of varying concentration of phosphatidylcholine on average vesicle size was studied. With increase in the phosphatidylcholine concentration the particle size increased the vesicle size. The effect of varying concentration ofphosphatidylcholine on vesicle size was determined. With increase in the polymer concentration the vesicle size increased. The Average vesicle size was found to be in the range of 220.14 ± 0.25 to 345.58 ± 0.41, the minimum vesicle size was found in formulation F-5, 220.14 ± 0.25 nm.

From the drug entrapment efficiency results, it is clear that drug entrapment efficiency of Invasomes formulations is reduced with lowering concentration of lacithin. This is because of the fact that low lecithin content provides less drug entrapment efficiency. The ability of

formulation to withstand drug molecules in the bilayer membrane of the vesicle was proves the encapsulation efficiency of Invasomes formulations. The Entrapment Efficiency of formulation F1. F2, F3, F4,F5 and F6 was found to be 65.58 ± 0.25, 68.54 ± 0.36, 63.32 ± 0.14, 65.56 ± 0.35, 76.65 ± 0.24 and 69.95 ± 0.18 percentage respectively. The maximum percentage entrapment efficiency was found to be in formulation F-5, 76.65 ± 0.24 percentages. The optimized formulation of invasomes was further incorporated into Carbopol gel base and evaluated for viscosity, pH, Spreadability and In Vitro drug release. The prepared gel at least rpm of 10 exhibited a viscosity of 3045 ± 14 to 3565 ± 15 cps that indicates that the formulation has the desired viscosity required for semisolid formulation for proper packaging. It was found that the viscosity decreases as the rotational speed of viscometer increased suggesting that greater the shearing the lower viscosity favours easy spreadability further confirmed by spreadability and rheological testing. pH of prepared herbal Gel was measured by using digital pH meter. The pH of theGel was found to be in range of 6.75 ± 0.02 to 6.98 ± 0.02 which is good for skin pH. All the formulation of Gel was shown pH nearer to skin required i.e. pH of IG1- 6.75 ± 0.02, IG2-6.82 ± 0.03 and IG3-6.98 ± 0.02. Spreadability plays considerable role inpatient compliance and ensures uniform application of Gel to a larger area of the skin. The spreadability of the formulation IG-2 was calculated as 10.23 ± 0.32cm/sec. The low value of spreadability coefficient of the Gel was sufficient suggesting easy spreading and no signs of grittiness. The lower value of spreadability indicates the lesser work required to spread the Gel over the skin, which means formulation was easily spreadable by applying small amount of shear. Drug content of tazarotene incorporated invasomes gel for formulation IG-1, IG-2 and IG-3 was found to be 96.65 ± 0.15**,** 99.12 ± 0.25 and 98.58 ± 0.32 respectively. The maximum drug content was found in formulation IG-2 (99.12 ± 0.25), select as optimized formulation.

CONCLUSION

In conclusion,the invasomes delivery systems may be a promising carrier for transdermal deliveryof tazarotene for the management of treatment of acne.

CONFLICTS OF INTEREST

There are no conflicts of interests.

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