

**PREPARATION AND IN-VITRO CHARACTERIZATION OF
MINOXIDIL INVASOMAL GEL FOR ALOPECIA****V. P. Sathvika*¹, Sudha B. S.², Dr. Shachindra L. Nargund³**

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ABSTRACT

Invasomes are flexible phospholipid-based nano vesicular systems developed to enhance transdermal drug delivery. They are composed of phospholipids, ethanol, and terpenes, which act synergistically to improve skin penetration. Terpenes disrupt the stratum corneum lipid structure, while ethanol increases vesicle deformability and fluidity. This unique composition enables effective delivery of both hydrophilic and lipophilic drugs. Invasomes overcome the major barrier of the skin and provide improved drug deposition in deeper layers. They offer advantages such as non-invasive administration, sustained release, and improved patient compliance. Invasomes have demonstrated potential in the treatment of alopecia, cancer, acne, hypertension, and immunological disorders. Overall, invasomes represent a promising nanocarrier system for advanced topical and transdermal drug delivery.

KEYWORDS: Invasomes, Minoxidil, Gel, Terpenes, Alopecia, thin film hydration.

INTRODUCTION

Invasomes are type of artificial vesicular nanocarrier that transport substances through the skin, the most superficial biological barrier. These are tiny, flexible bubbles made from fats and other natural ingredients. They are designed to help medicines pass through the skin

more easily. These are especially useful for treating skin problems and conditions like alopecia. They are made of three main parts: phosphatidylcholine, ethanol, and terpenes. These ingredients work together to make the skin more open to letting medicine through.^[1,2] There are two main ways invasomes help medicine get through the skin: they can squeeze through small gaps in the skin and can mix with the fats in the skin and break them apart, making it easier for the medicine to go deeper.

Ethanol softens both the skin and the invasomes, making them more flexible and better at moving through the skin. Terpenes also help by breaking up the tightly packed layers of fat in the skin, creating more space for the medicine to enter'. Invasomes shows deeper skin and follicular penetration when compared to ethosomes. Ethosomes lack terpenes and hence it require high ethanol concentration to achieve the same which can irritate the skin.^[3,4]

Androgenic alopecia is a hair related disease characterized by substantial hair fall in both male and female. Here the hair loss is progressive and leads to baldness. It affects at least 50% of men by the age of 50 years, and up to 70% of all males in later life. The hair loss is heritable, androgen-dependent, mediated by dihydrotestosterone and occurs in a defined pattern. Minoxidil is effective in the management of androgenic alopecia. It belongs to the vasodilator category. It was initially developed as an anti-hypertensive agent and was used orally in the treatment of hypertension since 1960s. The unwanted side effects of hypertrichosis in 100% of patients motivated its topical use in the treatment of hair loss.^[5]

Minoxidil enhances the anagen phase, resulting in increased hair length and thickness. It affects the potassium channel present in the vascular smooth muscles and hair follicles. The potassium channel activity may induce: Stimulation of micro circulation around the hair follicles induces arteriola vasodilation so it encourages conditions conducive to hair growth. Direct stimulation of the hair follicles as the drug acts as an epidermal growth factor on the matrix cells, slowing their aging process and extending their anagen phase. Gels are defined as semi rigid systems in which the movement of the dispersing medium is restricted by an interlacing three-dimensional network of particles or solvated macromolecules of the dispersed phase. Topical gels are more stable than creams and ointments, and can adhere well to the site of application.^[6,7]

Invasomal gel formulations help deliver medicines like minoxidil deeper into the skin in safe and comfortable way. They improve the drug is penetration and reduce the chance of skin

irritation. This makes them a better and more patient-friendly option for treating long-term hair loss problems like androgenetic alopecia.^[8] The present study focuses on developing a minoxidil-loaded Invasomal gel for topical application in the treatment of alopecia.

MATERIALS AND METHODS

Minoxidil was provided by Drug Testing Laboratory, Bengaluru. Soya lecithin Sisco research laboratories pvt ltd, Mumbai. Cholesterol Avra synthesis Pvt Ltd. Hyderabad. Terpene, Propylene Glycol Thomas Baker, Mumbai. Ethanol Avantor performance materials India ltd, Mumbai. Triethanolamine SDFCL, worll road, Mumbai. Carbopol 940 Central Drug House (P) Ltd, New Delhi.

METHODOLOGY

➤ Preformulation Studies

Preformulation testing is the first step in the development of dosage form of a drug substance. It can be defined as an investigation of physical and chemical properties of drug substance alone and when combined with excipients. The overall objective of the preformulation testing is to generate information useful to the formulator in developing stable dosage form, which can be mass-produced.

- 1. Identification of pure drug:** Weighed amount of drug was mixed with IR grade KBr (1: 10) and compressed under 10-ton pressure in a hydraulic press to form a transparent pellet. The pellet was scanned by IR spectrophotometer over a range of 4000-1 cm to 500-1 cm range.
- 2. Determination of Melting point:** Determination of melting point gives an idea about purity of the drug. Melting point of minoxidil was determined by capillary tube method. Fine powder of minoxidil was filled with glass capillary (previously sealed on one end). The capillary tube is tied to thermometer and the thermometer was kept in tube containing liquid paraffin. The Assembly was kept on heating temperature was allowed to increase gradually temperature at which the powder melts were noticed.
- 3. Determination of λ_{max} of minoxidil:** 10 mg of minoxidil dissolved in 100ml of methanol (100 $\mu\text{g/ml}$) standard solution¹ was prepared. From stock¹ 1ml was dissolved in 10ml of methanol (10 $\mu\text{g/ml}$). This solution was scanned using UV spectrometer from

200-400nm wavelength. The λ_{max} of minoxidil drug in methanol was found to be 286nm.

4. Preparation of Calibration Curve of minoxidil: 10mg of minoxidil was dissolved in 100ml methanol to obtain stock solution 1(100 μ g/ml). From this stock solution 1 further dilution was prepared by dissolving 0.2,0.4,0.6,0.8 and 1ml in 10ml of methanol to obtain 2,4,6,8 and 10 μ g/ml concentrations. The absorbance was measured against the phosphate buffer pH 7.4 as blank at 288 nm using UV spectrophotometer. The study was carried out in triplicate (n=3). Then the calibration curve was plotted taking concentration on X-axis and mean absorbance on Y-axis.

➤ **Preparation of minoxidil loaded invasomes by thin film hydration method**

Minoxidil invasomes were prepared by the thin film hydration method with slight modifications. Accurately weighed quantities of soya lecithin (200 mg) and minoxidil (100 mg) along with cineole (0.1 mL) were dissolved in 10 mL of chloroform and methanol mixture (2: 1 ratio). The solution was transferred into a round bottom flask and subjected to rotary evaporation for 2 hours to remove the organic solvent and to form a thin lipid film on the inner wall of the flask. The formed thin film was kept overnight to ensure complete removal of residual organic solvent. The dried lipid film was then hydrated with 15 mL of hydration medium consisting of phosphate buffer pH 7.4 and ethanol in the ratio of 7: 3 for 30 minutes with gentle rotation. The resulting dispersion was further sonicated for 30 minutes to reduce the vesicle size and obtain a uniform invasomal formulation containing minoxidil.

Table 1: Formulation of drug loaded invasomes.

Formulation Code	Minoxidil (w/v)	Soyalecithin (w/v)	Cineole (v/v)	Lemonene (v/v)	Cholesterol (w/v)	Chloroform : methanol (2: 1)
F1	1%	2%	1%	-	0.5%	10ml
F2	1%	1.75%	1%	-	0.5%	10ml
F3	1%	1.5%	1%	-	0.5%	10ml
F4	1%	2%	0.5%	-	0.5%	10ml
F5	1%	1.75%	0.5%	-	0.5%	10ml
F6	1%	1.5%	0.5%	-	0.5%	10ml
F7	1%	2%	-	1%	0.5%	10ml
F8	1%	1.75%	-	1%	0.5%	10ml
F9	1%	1.5%	-	1%	0.5%	10ml
F10	1%	2%	-	0.5%	0.5%	10ml
F11	1%	1.75%	-	0.5%	0.5%	10ml
F12	1%	1.5%	-	0.5%	0.5%	10ml

Table 2: Process variables trials of minoxidil invasomes.

FORMULATION CODE	MINOXIDIL (w/v)	SOYALECITHIN (w/v)	CINEOLE (v/v)	CHOLESTEROL (w/v)	CHLOROFORM: METHANOL (2: 1)
F3(optimized formulation) - Temp-50⁰C - Hydration time-30mins - Hydration solution-ethanolic PBS 7.4pH(7: 3ratio) - Sonication time- 30mins	1%	1.5%	1%	0.5%	10ml
Influence of temperature					
F13 Temp- 60 ⁰ c	1%	1.5%	1%	0.5%	10ml
Influence of hydration time					
F14 Hydration time-60mins	1%	1.5%	1%	0.5%	10ml
Influence of hydration solution					
F15 Hydration solution- PBS 7.4pH	1%	1.5%	1%	0.5%	10ml
Influence of sonication time					
F16 Sonication time-60mins	1%	1.5%	1%	0.5%	10ml

➤ Formulation of minoxidil invasomal gel

Gel was prepared by using Carbopol 940 as a gelling agent. Weight specified quantity of Carbopol 940 and placed in a beaker containing purified water and allowed to soak for 12h (overnight) and neutralized by adding Triethanolamine. To this, Minoxidil-loaded invasomal suspension was added slowly with gentle continuous stirring until a homogeneous gel was formed.

Table 3: Formulation chart of minoxidil invasomal gel.

Formulation code	Carbopol 934	TEA	Methyl paraben	MXD invaomes equivalent to MXD	Distilled water
Minoxidil gel (F3)	125mg	2ml	10mg	25ml	25ml

➤ Post formulation studies

➤ Evaluation of Nanoparticles

1. Particle size: The average Particle size was evaluated in Horiba Scientific (Nanopartica) SZ- 100. Before the measurement, the invasomal suspension was allowed to Sonication for 30mins and taken 1ml of suspension was diluted with double distilled water and poured into the polystyrene cuvettes kept inside the instrument and measured the sample of Particle size & PDI n=3.

2. Zeta- potential: The surface charge (zeta potential) was determined by measuring the electronic mobility of the particles. It is a useful parameter to predict the physical stability of a colloidal system. 1ml of the prepared invasome suspension was diluted with 10ml of distilled water. Then the samples were transferred into the cuvette which contains electrodes and kept inside the instrument. Then the laser scanning light was passed through the cuvette tube and analysed the zeta potential using Horiba scientific (Nanoparticle) SZ-100. The test was repeated 3 times (n=3) the mean of those readings was noted down.

3. Drug content: 1ml of the prepared invasomal suspension was taken in 10ml volumetric flask was diluted using methanol and was sonicated for 10 mins. Further dilutions were prepared using phosphate buffer 7.4 and absorbance was measured using uv spectrophotometer at 286nm.

4. Entrapment efficiency: Entrapment efficiency is defined as the portion of the applied drug that is entrapped by the invasomes. Un encapsulated free drug can be removed from the invasomal solution using centrifugation. The entrapment efficiency was determined by measuring the concentration of the drug in the Supernatant liquid after centrifugation. Minoxidil invasomal suspension 5ml was taken into the centrifugation tube. This suspension was centrifuged at 7000 rpm for 30min at 40°C. Withdrawn clear supernatant using micro pippete and diluted suitable with ethanol and measured spectrophotometrically at 286 nm. The entrapment efficacy was calculated by using the following equation. The amount of drug in supernatant (W_{free}) was then subtracted from the total amount of drug of the preparation (W_{total}). Then the test was repeated 3 times (n=3) the mean of those reading was noted down.

$$\text{Entrapment Efficacy} = \frac{W_{\text{total}} - W_{\text{free}}}{W_{\text{total}}} * 100.$$

5. In-vitro drug release studies: The In vitro release of the drug from Minoxidil invasome formulation was investigated in phosphate buffer (pH=7.4) solution using the dialysis bag (regular dialysis) method. Which is regenerated with seamless cellulose tubing, membranes

are partially permeable and have molecular weight cut off between 12000-14000 Da. Pore size is 2.4 nanometres; ideal for filtration and diffusion work, which overnight in pH 7.4 phosphate buffer. Soaking the membrane in water or buffer overnight allows it to become fully hydrated, achieving its optical porosity and flexibility. Overnight soaking allows it to become fully hydrated, achieving its optimal dialysis membrane. This is essential for proper diffusion of solutes across the membrane, which could alter its permeability. Minoxidil loaded invasomal suspension equivalent to 10mg of the total drug used was taken in a dialysis bag and the bag was placed in a beaker containing 200ml of PBS pH 7.4, which acted as receptor compartment. The temperature of the receptor medium was maintained at $37\pm^{\circ}\text{C}$ and agitated using a magnetic stirrer with the speed of 100 rpm. Aliquots of 2 ml sample were photo periodically and after each withdrawal, the same volume of medium was replaced. The collected samples were analyzed using a UV spectrophotometer at 255 nm. The tests were carried out in triplicates ($n=3$).

➤ Evaluation of gel

1. Measurement of pH: The pH of gel formulations was determined by a digital pH meter. 1g of gel is dissolved in 25 ml distilled water and stored for two hours. The measurement of the pH of each formulation was done in triplicate and average values were considered.

2. Drug content studies: Accurately weighed quantity of invasomal gel was transferred into a 10 ml volumetric flask and volume made up to 10 ml using ethanol and stirred for 30 min followed by a magnetic stirrer. 1 ml of the above solution was further diluted to 10 ml with ethanol. The absorbance was measured against the blank as ethanol using a Shimadzu 1800 UV Visible spectrophotometer at 286nm.

3. Viscosity measurement: Viscosity of the gel was determined by using Brookfield viscometer. Accurately weighed 25gm minoxidil invasomal loaded gel was transferred to 50 ml glass beaker. Spindle no 63 was selected and it is immersed into the gel. The viscometer was operated at 10 rpm until the reading gets stabilized and reading was noted in centipoises.

4. Spreadability: The spreadability of Minoxidil invasomal gel was determined by pressing 1 g of gel in transparent circular glass slides. Initially, mark the place where the gel is placed in a glass slide, and allow maximum spreading for 5 mins by placing 400gm weights. The diameter of the formed circle was measured to express the spreadability. In these cases, spread ability m is determined by the formula,

$$A = \pi r^2$$

$$Si = (d^2 \times \pi / 4)$$

Where,

Si- spreading area(mm²) depending on mass d- spreading area diameter(mm)

5. In-vitro diffusion studies: An in-vitro diffusion study was carried out in a Franz diffusion cell using a dialysis membrane. Care should be taken to avoid the entry of air bubbles below the dialysis membrane throughout the study and the membrane was soaked overnight in pH 7.4 phosphate buffer. The membrane was placed in between the donor and reservoir compartment containing 200 ml of pH 7.4 phosphate buffer in the receptor compartment. Weighed minoxidil invasomal gel equivalent to 10mg of the drug and placed it over the dialysis membrane of the donor compartment. The whole set was placed on the magnetic stirrer; the study was carried out at $37 \pm 0.5^\circ\text{C}$ with 100 rpm. Samples were withdrawn from the sampling port of the reservoir compartment at regular intervals and the same volume was replaced and absorbance was measured using a Shimadzu 1800 UV visible spectrophotometer at 286 nm.

- **Release kinetics**

- a. **Zero order kinetics**

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation

$$Q_t = Q_0 + K_0 t$$

Where, Q_t = Amount of drug dissolved in time t , Q_0 = Initial amount of drug in the solution ,
 K_0 = Zero order release constant

- b. **First order kinetics**

To study the first order release rate kinetics the release rate data were fitted to following equation.

$$\log Q_t = \log Q_0 + K_t / 2.303$$

Where, Q_t = Amount of drug released in time t

- c. **Higuchi Model**

Graph was plotted between cumulative percentages of drug released vs. square root of time.

$$Q = Kt/2$$

Where, K= the constant reflecting the design variables of the system and t is the time in hours.

d. Korsemeyer-Peppas model (Power Law)

The power law describes the drug release from the polymeric system in which release deviates from Fickian diffusion, as expressed in the following equation.

$$M_t / M_\infty = k t^n \quad \log [M_t / M_\infty] = \log k + n \log t$$

Where, M_t and M_∞ are cumulative amounts of drug released at time t and infinite time (i.e., fraction of drug release at time t), k = constant, n = diffusion release exponents indicative of the mechanism of drug release.

6. Stability Studies: Stability of a drug has been defined as the stability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications throughout its shelf life.

Procedure: The Optimized (F14) of minoxidil invasomes was carried out according to the ICH Q1C for zones III and IV (International Conference on Harmonization). Minoxidil invasomal suspension was stored at room temperature and refrigerator for one months to check the stability of the formulation. The formulation's alterations were tracked by measuring particle size, PDI, ZP, and EE 1months of storage 37°C. The samples were collected at regular intervals of the first, second, and third months and evaluated for pH and, drug content.^{[50],[13]}

RESULTS AND DISCUSSION

➤ Preformulation Studies

1. FT-IR spectroscopy of minoxidil

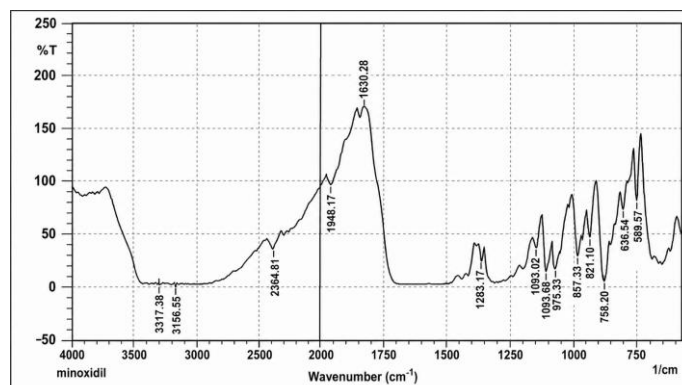


Figure 1: IR Spectrum of Minoxidil.

SL. NO	Vibrations	Standard Range (cm ⁻¹)	Observed peak (pure drug) (cm ⁻¹)
1	N-H Stretching	3300–3500	3317.69, 3156.97
2	C=N Stretching	1600–1650	1630.28
3	C-N Stretching	1200–1350	1283.17
4	N→O Stretching	1000–1100	1093.02
5	C-N / Ring vibration	950–1000	975.33

The identity and purity of Minoxidil were confirmed by melting point determination, UV spectroscopy and FTIR spectroscopy. The FTIR spectrum of the pure drug showed characteristic peaks corresponding to the major functional groups of Minoxidil. The observed peaks were at 3317.69 and 3156.97 cm⁻¹ for N-H stretching, 1630.28 cm⁻¹ for C=N stretching, 1283.17 cm⁻¹ for C-N stretching, 1093.02 cm⁻¹ for N→O stretching and 975.33 cm⁻¹ for C-N/ring vibration. These peaks were within the respective standard ranges, confirming the identity of the drug. The melting point of Minoxidil was found to be **247–248°C**, which was consistent with the expected melting point range of the drug and further supported its identity and purity.

2. Calibration Curve of Minoxidil

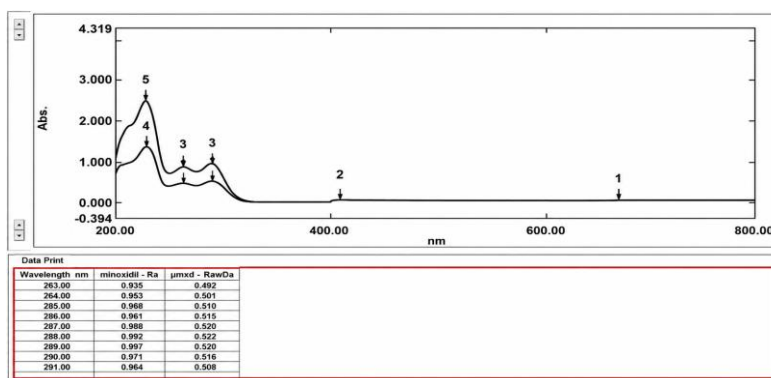


Figure 2: λ_{max} of Minoxidil in methanol.

Sl.NO	Concentration ($\mu\text{g/ml}$)	Absorbance(n=3)
1	0	0
2	2	0.178 $\pm$ 0.0014
3	4	0.297 $\pm$ 0.0021
4	6	0.445 $\pm$ 0.0009
5	8	0.668 $\pm$ 0.003
6	10	0.886 $\pm$ 0.004

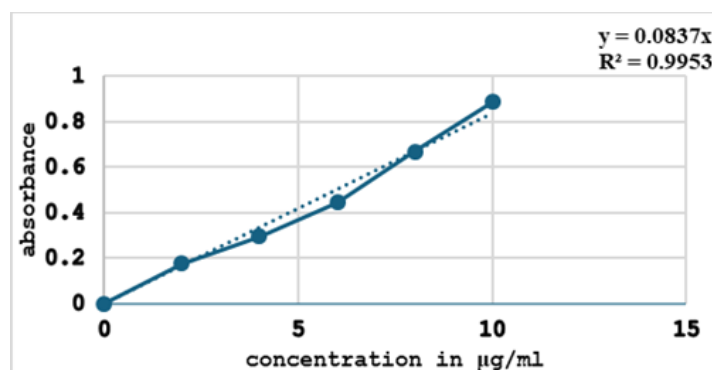


Figure 3: Calibration Curve of Minoxidil in methanol.

The UV spectrum of Minoxidil in methanol showed a maximum absorption wavelength (λ_{max}) at **286 nm**. The calibration curve demonstrated a linear relationship between concentration and absorbance over the concentration range of **2–10 $\mu\text{g/mL}$** . The regression coefficient (R^2) was **0.9953**, with a slope of **0.0837**, indicating good linearity of the analytical method. The observed absorbance values increased progressively with increasing drug concentration, confirming the suitability of the method for quantitative estimation of Minoxidil.

3. Drug–Excipient Compatibility Study

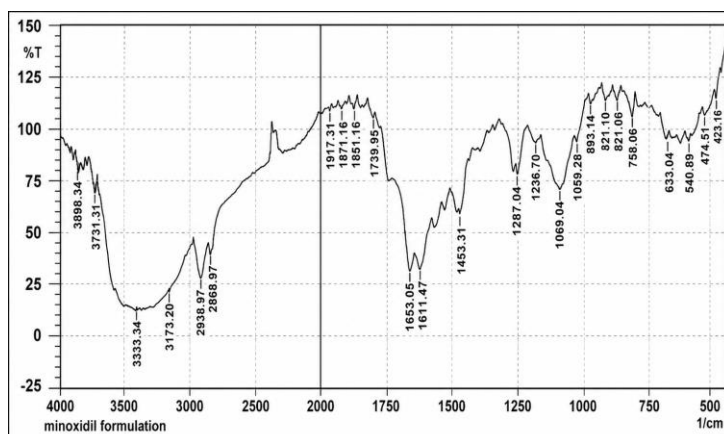


Figure 4: Observed IR peaks of pure drug and excipients.

SL. NO	Functional group	Standard Range (cm ⁻¹)	Observed peak (pure drug) (cm ⁻¹)	Observed peak (drug+excipient)
1	N–H stretching	3300–3500	3317.38	3333.34
2	N–H stretching	3300–3500	3156.55	3173.20
3	C=N stretching	1600–1650	1630.28	1633.11
4	C–N stretching	1200–1350	1283.17	1287.04
5	N→O stretching	1000–1100	1093.02	1069

FTIR analysis was performed to investigate possible interactions between Minoxidil and the formulation excipients. The characteristic peaks of Minoxidil were retained in the drug–excipient mixture, with only minor changes in peak positions. The N–H, C=N, C–N and N→O stretching vibrations remained identifiable. This indicated the absence of significant chemical interaction between Minoxidil and the lipid/excipient components used in the formulation.

The preservation of the characteristic functional-group peaks suggests that Minoxidil remained chemically stable in the presence of the selected excipients and supports their suitability for preparation of the invasomal formulation.

➤ Evaluation of Minoxidil-Loaded Invasomes

Minoxidil-loaded invasomes were prepared using the thin-film hydration method by varying the concentrations of soya lecithin, terpene and the type of terpene. A process variation was subsequently applied to the selected formulation. The prepared formulations were evaluated for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency and drug content.

Table 4: Evaluation of Minoxidil-Loaded Invasomes.

FORMULATION CODE	PARTICLE SIZE (nm)	ZETA POTENTIAL (-mV)	PI	%ENTRAPMENT EFFICIENCY	%DRUG CONTENT
F1	643.7	-14	0.818	95.7	91
F2	322.3	-26.9	0.859	95.6	89
F3	142.2	-31.1	0.246	88.4	88.4
F4	666.2	-17.7	0.885	96.6	93
F5	499.4	-29.2	0.419	95.16	94
F6	302	-33.3	0.805	94.4	92.4
F7	875.6	-13.2	0.865	89.77	89
F8	488.2	-26.8	0.630	88.1	85
F9	309	-32.6	0.427	75	73.2
F10	896.9	-17.9	0.094	89.2	85
F11	793.6	-26.9	1.24	87.6	79.1
F12	606.8	-34.4	0.066	78.5	77.6
F13	308.4	0.655	-28.2	89.6	89.3
F14	106	0.670	-32	92	91.3
F15	406.2	0.876	-30.6	92.1	91.3
F16	697	0.865	-28.6	86.9	85.3

Peak No.	Zeta Potential (mV)	Electrophoretic Mobility (cm ² /Vs)	Conductivity (mS/cm)
1	-31.1 mV	-0.000241 cm ² /Vs	0.0104
2	-- mV	-- cm ² /Vs	--
3	-- mV	-- cm ² /Vs	--
Total	-31.1 mV	-0.000241 cm ² /Vs	0.0104

Zeta Potential (Mean)

: -31.1 mV

Electrophoretic Mobility Mean

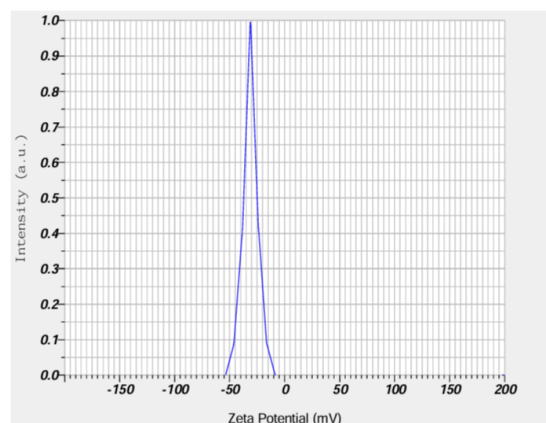
: -0.000241 cm²/Vs

Figure 5: Zeta potential of F3 formulation.

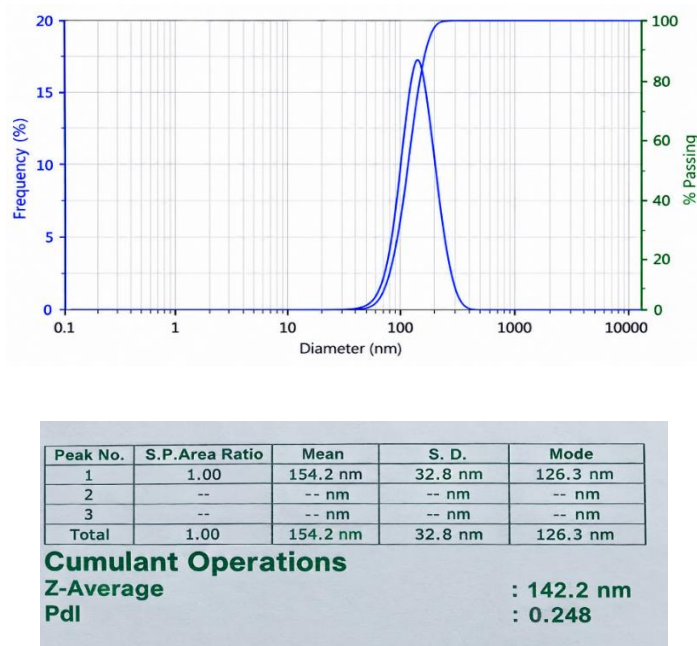


Figure 6: Particle size of F3 formulation.

The particle size of the prepared formulations ranged from **142.2 to 896.9 nm**, while the zeta potential ranged from **−13.2 to −34.4 mV**. The PDI values varied from **0.066 to 1.24**, and the entrapment efficiency ranged from **75.0 to 96.6%**.

- **Effect of Soya Lecithin and Terpene Variables on Invasomes**

The concentration and type of soya lecithin and terpene significantly influenced the physicochemical characteristics of the invasomes. Reduction of soya lecithin concentration from 2% to 1.5% affected particle size, PDI, zeta potential, entrapment efficiency, and drug content. Lower lipid concentration may provide insufficient lipid material for uniform vesicle formation and surface coverage. This reduced coverage may expose surface charges, resulting in a more negative zeta potential and greater variation in vesicle size. It may also reduce the capacity of vesicles to incorporate and retain Minoxidil, thereby lowering entrapment efficiency and drug content.^[55,60] Terpene concentration influenced vesicle size by altering bilayer fluidity, with higher concentrations generally promoting smaller vesicles. Lower terpene concentration may decrease membrane fluidity and produce relatively larger and more rigid vesicles. The reduced fluidity may enhance Minoxidil retention within the vesicles, thereby improving entrapment efficiency and drug content.^[2,9] The type of terpene also affected vesicle formation, with limonene potentially producing larger vesicles than cineole due to its greater hydrophobicity and bulkier structure. Overall,

both terpene concentration and type, together with soya lecithin concentration, influenced vesicle size, surface characteristics, and drug entrapment.^[61]

- **Selection of Optimized Formulation**

Among the prepared formulations, **F3** was selected as the optimized formulation based on its overall vesicular characteristics. F3 exhibited a particle size of **142.2 nm**, PDI of **0.246**, zeta potential of **−31.1 mV**, entrapment efficiency of **88.4%** and drug content of **88.4%**.

Although some other formulations showed slightly higher entrapment efficiency and drug content, F3 demonstrated a more favourable combination of small particle size, relatively narrow size distribution and adequate surface charge. The PDI value of 0.246 indicated comparatively good particle-size uniformity, while the zeta potential of −31.1 mV suggested sufficient electrostatic repulsion between vesicles. Therefore, F3 was selected as the optimized formulation based on the overall balance of critical formulation parameters.

- **Effect of Process Variables**

Effect of Temperature: Increasing the preparation temperature influenced particle size, PDI and zeta potential. Higher temperature increases particle kinetic energy and can soften the lipid matrix, thereby promoting particle collision and coalescence. Thermal stress may also result in a wider particle-size distribution. At the same time, heating may increase drug solubility within the lipid phase and facilitate incorporation of Minoxidil into the vesicular structure, resulting in increased entrapment efficiency and drug content.^[62]

Effect of Hydration Time: Increasing hydration time allows more complete hydration and rearrangement of the lipid film, facilitating vesicle formation. Prolonged hydration may improve dispersion of the lipid material and increase recovery of the formulation from the vessel. Consequently, increased hydration time may improve drug incorporation and entrapment efficiency.^[63,64]

Effect of Ethanol: Ethanol plays an important role in modifying the vesicular membrane. It can increase membrane flexibility and facilitate formation of smaller vesicles with improved uniformity. Removal of ethanol may therefore result in larger and less uniform vesicles with altered zeta potential. However, Minoxidil has high solubility in ethanol. The presence of ethanol may consequently increase partitioning of the drug into the external ethanolic phase and promote drug leakage from the vesicles. In the absence of ethanol, lower drug solubility

in the external aqueous phase may favour retention of Minoxidil within the vesicular structure and improve recovered drug content.^[65,66]

Effect of Sonication Time: Increasing sonication time can introduce excessive mechanical and thermal energy into the formulation. Prolonged cavitation may increase vesicle collision and aggregation, disturb the vesicular membrane and produce a wider particle-size distribution. Excessive sonication may also increase membrane permeability and facilitate leakage of entrapped Minoxidil. Thus, prolonged sonication may adversely affect particle size, PDI, zeta potential and entrapment efficiency, indicating that optimization of sonication time is important for maintaining vesicular integrity.^[67]

➤ Evaluation of Minoxidil Invasomal Gel

Table 5: Evaluation of Minoxidil Invasomal Gel.

Sl. No.	Parameter	Result
1	Appearance	Off-white
2	Homogeneity	Good
3	Spread ability	12.16±0.135
3	pH	6.26 ± 0.165
4	Percent drug content	91.40 ± 0.815
5	Viscosity (cps)	3802

The optimized Minoxidil invasomal formulation was incorporated into Carbopol 934 gel and evaluated for appearance, homogeneity, spreadability, pH, drug content and viscosity. The prepared gel showed an **off-white appearance and good homogeneity**. The measured pH was **6.26 ± 0.165**, indicating compatibility with the reported scalp pH range. The viscosity was **3802 cps**, while the drug content reported in the results was **91.40 ± 0.815%** and spreadability was **12.16 ± 0.135**. The relatively suitable pH of the formulation and its good homogeneity indicate that the invasomal vesicles could be incorporated into the Carbopol gel system without producing visible instability.^[68] The viscosity provided sufficient consistency for topical application while allowing the formulation to spread over the application site.

➤ *In-vitro* diffusion studies of minoxidil invasomal gel

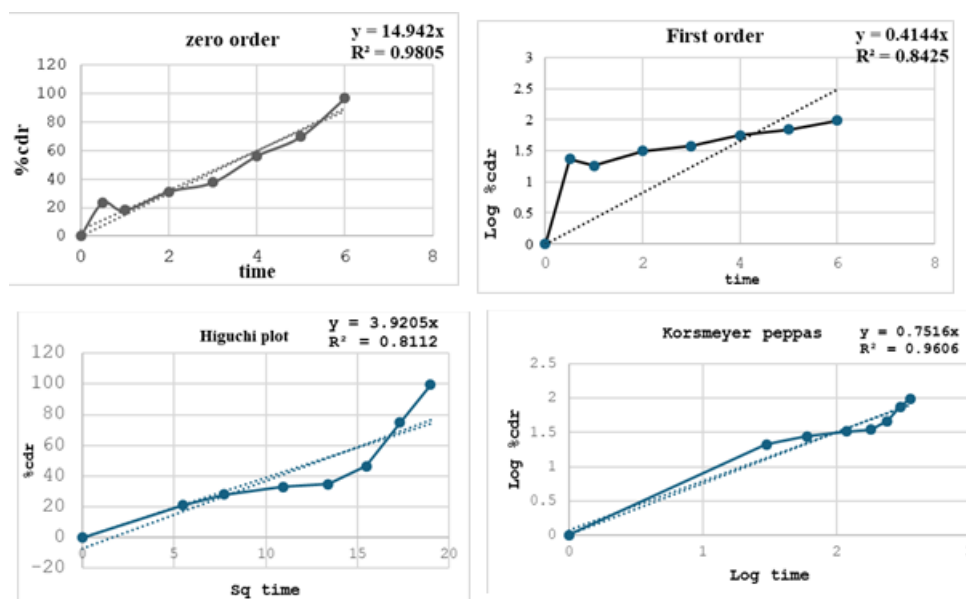


Figure 7: In-vitro diffusion studies of minoxidil invasomal suspension.

The in-vitro drug-release data of the optimized Minoxidil invasomal suspension and invasomal gel were fitted to zero-order, first-order, Higuchi and Korsmeyer–Peppas models. For the optimized invasomal suspension, the regression coefficients were **0.9805**, **0.8425**, **0.9498** and **0.9948** for zero-order, first-order, Higuchi and Korsmeyer–Peppas models, respectively. The Peppas exponent (n) was **0.7473**, indicating a **non-Fickian release mechanism**.

For the Minoxidil invasomal gel, the corresponding regression coefficients were **0.9649**, **0.8323**, **0.9381** and **0.9944**, respectively, with a Peppas exponent of **0.7516**, also indicating **non-Fickian release**.

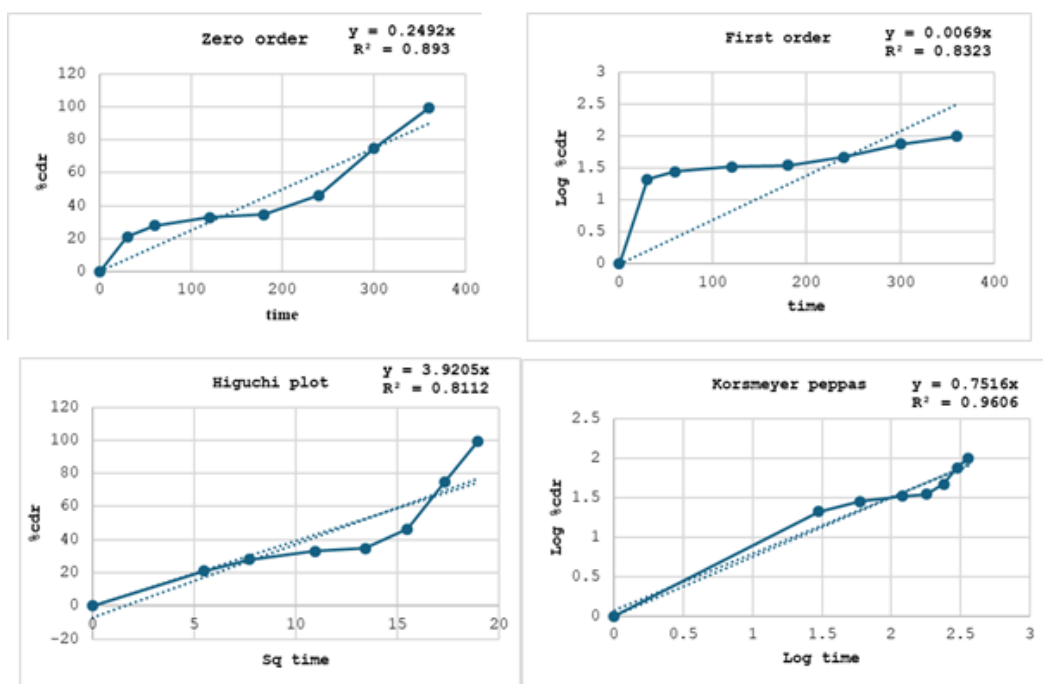


Figure 8: *In-vitro* diffusion studies of minoxidil invasomal gel.

The higher release observed from the invasomal suspension compared with the gel may be attributed to the lower viscosity of the suspension, which facilitates movement of the entrapped drug into the dissolution medium. Incorporation into the Carbopol gel increases viscosity and consequently restricts drug mobility and diffusion, resulting in comparatively slower drug release. The zero-order behaviour indicates relatively controlled drug release over time, whereas the non-Fickian release mechanism suggests that drug release involves more than simple diffusion and is influenced by the polymeric/vesicular matrix.

- **Stability Studies:** The optimized invasomal formulation was subjected to stability studies at refrigerated conditions ($2-8^{\circ}\text{C}$) and room temperature ($30 \pm 2^{\circ}\text{C}$) for one month. At refrigerated conditions, no significant changes were observed in the evaluated parameters. At room temperature, an increase in particle size and a decrease in entrapment efficiency were observed during storage.

Table 6: Stability studies invasomes.

OPT (F14)	Particle Size(nm)		Zeta Potential (mV)		Entrapment Efficiency (%)	
Days	At RT 25°C	At RF $2-8^{\circ}\text{C}$	At RT 25°C	At RF $2-8^{\circ}\text{C}$	At RT 25°C	At RF $2-8^{\circ}\text{C}$
0	150-247	168-260	-31.1 $\pm$ 0.25	-32.1 $\pm$ 0.2	95.1 $\pm$ 2.15	91.45 $\pm$ 2.5
10	253-385	295-379	-26.5 $\pm$ 0.31	-29.8 $\pm$ 0.2	88.15 $\pm$ 2.3	88.13 $\pm$ 3.1
20	498-650	471-582	-21.2 $\pm$ 0.28	-20.6 $\pm$ 0.34	87.5 $\pm$ 3.1	85.75 $\pm$ 2.8
30	666-896	614-813	-17.8 $\pm$ 0.24	-16.4 $\pm$ 0.25	77 $\pm$ 2.5	75.49 $\pm$ 2.12

*RT: Room temperature, RF: Refrigerator

The particle size increased progressively during storage at both temperatures, with a more pronounced change at room temperature. The zeta potential also became less negative with increasing storage time, while entrapment efficiency decreased. These changes suggest gradual vesicular aggregation and reduced physical stability during storage, particularly at higher temperature. The observed sedimentation and coagulation at room temperature further support the influence of temperature on vesicular stability. The results therefore indicate that refrigerated storage at 2–8°C provided better maintenance of the physical characteristics of the optimized invasomal formulation during the one-month study period.

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