FORMULATION AND EVALUATION OF CUBOSOMES LOADED WITH MINOXIDIL AND SAW PALMETTO OIL FOR PREVENTION OF HAIRLOSS

A dissertation submitted to

The Tamil Nadu Dr. M. G. R. Medical University, Chennai-600 032

In partial fulfilment of requirements for the award of the degree of

BACHELOR OF PHARMACY

2020-2024

Submitted by

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CERTIFICATE

This is to certify that this dissertation work entitled **"Formulation and evaluation of cubosomes loaded with minoxidil and saw palmetto oil for preventing hair loss"** submitted by **GOKUL D (560020511515), MOHAMED MUFEES E M (560020511546), SANDEEP G (560020511577), MUGESHKUMAR K (560021511601),** in partial fulfilment of the degree of Bachelor of Pharmacy under The Tamil Nadu Dr. M.G.R. Medical University. Chennai, under my direct supervision and guidance during the academic year 2024-2025.

Place: Coimbatore
Date:

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Place: Coimbatore **Dr. M. Ramanathan, D.Sc,**

Date: **PRINCIPAL**

EVALUATION CERTIFICATE

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Examination Centre: PSG College of Pharmacy, Coimbatore – 04

Date:

INTERNAL EXAMINER **EXTERNAL EXAMINER**

DECLARATION

We hereby declare that this dissertation entitled **"Formulation and evaluation of cubosomes loaded with minoxidil and saw palmetto oil for preventing hair loss"** was carried out by us, under the guidance of **Mrs. R. Nithya, M.Pharm., Assistant professor, Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore-641004.**

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INTRODUCTION

LIPID DRUG DELIVERY SYSTEM

Lipid-based drug delivery systems (LDDS) have emerged as a promising approach for enhancing the solubility, stability, and bioavailability of both hydrophilic and hydrophobic drugs. These systems utilize lipid carriers to improve drug absorption and targeted delivery, minimizing systemic toxicity.

LDDS can be classified into various types, including liposomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), self-emulsifying drug delivery systems (SEDDS), lipid micelles, cubosomes, lipid-based emulsions, and phytosomes.(1)

Advantages of LDDS:

- Enhanced bioavailability and solubility of poorly water-soluble drugs.
- Controlled and sustained drug release for prolonged therapeutic effects.
- Protection of drugs from degradation and enzymatic metabolism.
- Biocompatibility and reduced systemic toxicity.
- Versatility for multiple administration routes (oral, topical, parenteral, etc.).

Disadvantages of LDDS:

- Complex formulation processes requiring precise control over particle size and composition.
- Stability issues, including susceptibility to oxidation and hydrolysis.
- High cost of production and specialized manufacturing techniques.
- Potential batch-to-batch variability affecting product consistency.

CUBOSOMES

Lipid-based vesicular systems, such as cubosomes, have emerged as promising nanocarriers in drug delivery applications due to their unique structural and physicochemical properties. Cubosomes are self-assembled lipid vesicles that share similarities with conventional vesicular systems like liposomes but exhibit a distinctive bicontinuous cubic phase structure. These nanostructures are typically formulated using specific amphiphilic lipids in the presence of an appropriate stabilizer, enabling the formation of thermodynamically stable dispersions. Since their discovery, cubosomes have garnered significant interest as advanced drug delivery systems due to their high surface area, ability to encapsulate diverse bioactive compounds (hydrophilic, hydrophobic, and amphiphilic), and controlled drug release properties.

Cubosomes have been explored for a variety of drug delivery routes, including oral, ocular, transdermal, and chemotherapeutic applications. Their potential in cancer therapeutics is particularly noteworthy, as their nanostructured cubic phase facilitates enhanced drug solubilization, prolonged release, and targeted delivery. The ease of their fabrication further enhances their applicability, with common preparation techniques including emulsification, sonication, and homogenization.

Advantages of Cubosomes:

- Biocompatible, biodegradable, and non-irritating
- Thermodynamically stable
- High drug-loading capacity due to large interior surface area
- 3D nanostructure with hydrophilic and hydrophobic domains for versatile drug delivery
- Complex diffusion pathway enables sustained drug release
- Simple preparation process
- Superior solubilization compared to other lipid-based carriers
- Enhances bioavailability of water-soluble peptides

Disadvantages of Cubosomes:

- Limited entrapment of water-soluble drugs due to high water content
- Challenges in large-scale production due to high viscosity
- Risk of drug leakage during storage or in vivo transmission
- Susceptible to phase transitions upon environmental exposure
- Potential for particle growth over extended storage periods.

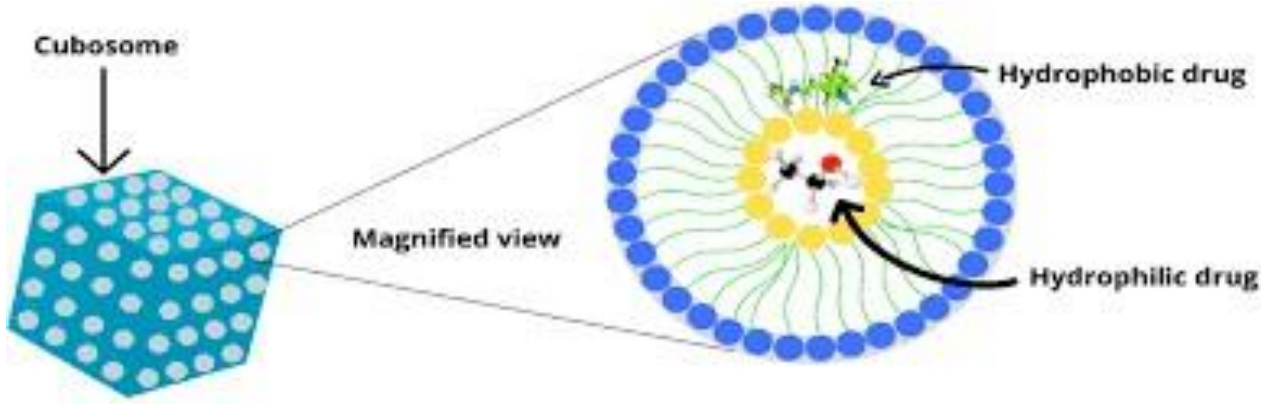


Figure 1: Structure of cubosome(2)

Mechanism of action:

The drug release mechanism from cubosomes is based on the principle of drug diffusion, where the concentration gradient of the drug across the cubosomes is the driving force of the diffusion. Therefore, the drug release rate from cubosomes is generally coincidental with the Higuchi or Fick diffusion equation.

There are many factors influencing the drug release rate, such as drug solubility, diffusion coefficient, partition coefficient, cubic liquid-crystalline geometry, pore size and distribution, interface curvature, temperature, pH, and ionic strength of the release medium.

The release mechanism of several hydrophilic model drugs from the cubic and reversed hexagonal liquid crystalline was investigated. These studies indicated that diffusion is the

predominant mechanism of drug release, and the drug release rate from cubic ones is faster than the hexagonal liquid crystalline.

But it is difficult for the hydrophobic drug to escape from the cubosomes in vitro due to the affinity of the drug with the hydrophobic domain in the cubic phase.

Preparation of Cubosomes:

Cubosomes are prepared by two distinct methods.

1.Top-Down method

2.Bottom-up method

1. Top-Down method:

The most common approach for producing cubosomes is the top-down method, which involves two parts. The first step is to make a viscous bulk cubic phase by combining lipid with a stabiliser to prevent aggregation; the second step is to disperse the previous steps resulting in an aqueous medium using high-energy methods like high-pressure homogenisation or sonication, finally generating cubosomes. (2)

2. Bottom-Up method:

The bottom-up process starts with the creation of nanostructure basic building blocks, which are then put together to create the final product. It is a more recently established method of cubosome production. Cubosomes can also be created at room temperature via a technique known as crystallisation from precursors. This procedure is referred to as the liquid precursor or solvent dilution method, according to Spicer et al. To make discrete nanoparticles, a polymer, a liquid crystal-forming lipid, and a hydrotrope are dispersed in surplus water with low energy input. Hydrotrope is a vital component of the bottom-up strategy because it may prevent the formation of liquid crystals at high concentrations and break down water-insoluble lipids to make liquid precursors. Unlike the top-down technique, this dilution-based procedure can produce cubosomes without the requirement for time-consuming fragmentation. (3)

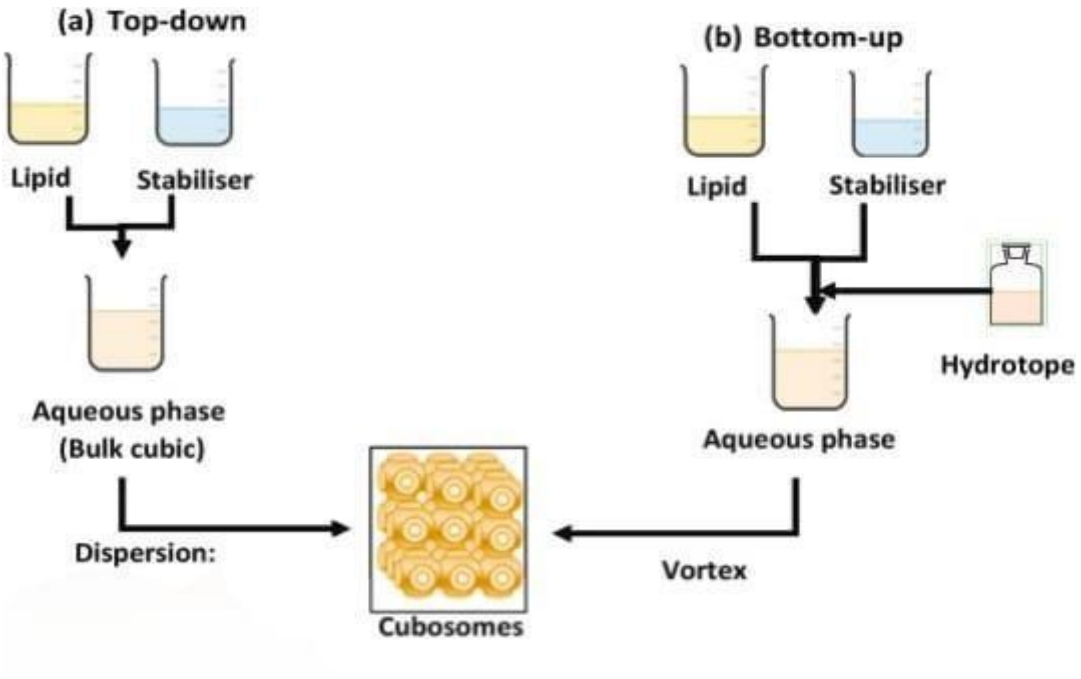


Figure 2: preparation method of cubosome(4)

Examples of drugs loaded in cubosomes for treating various diseases:

Drug	Delivery system	Application	Lipid composition
Rapamycin	Topical gel	Psoriasis treatment	GMO
Latanoprost	Eye drops	Glaucoma management	GMO
Fluconazole	Anti-fungal cream	Cutaneous cadidiasis	GMO
Insulin	Oral delivery system	Sustained delivery of insulin	Phytantriol
Erythromycin	Topical gel	Treatment of skin bacterial infection	GMO

Table 1: Examples of drugs loaded in cubosomes for treating various diseases

MINOXIDIL

Minoxidil is a widely used topical drug for hair regrowth and is approved by the FDA for treating androgenic alopecia. Originally developed as an antihypertensive medication, its hair growth properties were discovered as a side effect, leading to its formulation as a topical treatment for hair loss.

Role in hair growth:

- ATP-Sensitive Potassium Channel Activation– Opens K+ channels, leading to follicular cell hyperpolarization and enhanced hair follicle activity.
- Vasodilation and Increased Blood Flow – Expands peripheral blood vessels, improving oxygen and nutrient supply to hair follicles.
- Prolongation of the Anagen Phase– Extends the active growth phase of the hair cycle, delaying follicular miniaturization(5)

Advantages:

- Clinically Proven for Hair Regrowth
- Non-Hormonal Action: Unlike finasteride, it does not alter DHT levels, making it safer for both men and women.
- Widely Available: Easily accessible in 2% and 5% formulations for topical use.
- Effective for Different Types of Hair Loss: Useful for androgenic alopecia, alopecia areata, and telogen effluvium**.**

Disadvantages of minoxidil:

- Requires Long-Term Use: Hair regrowth stops if treatment is discontinued.
- Initial Shedding Phase: Temporary hair shedding occurs as weaker hairs fall out before new growth begins.
- Side Effects: Can cause scalp irritation, dryness, itching, and redness in some users.
- Limited Effectiveness in Advanced Baldness: Works best in early-stage hair loss and has less impact on completely bald areas**.**

Delivery systems of minoxidil:

1. **Conventional Topical Solutions** – The most commonly used liquid-based minoxidil, often containing alcohol and propylene glycol for penetration.(6)
2. **Nanocarrier-Based Systems** – Advanced delivery using liposomes, nano emulsions, and nanoparticles to improve scalp absorption.(11)
3. **Oral Minoxidil (Low-Dose)** – Emerging as an alternative for patients who do not respond to topical minoxidil.(7)

SAW PALMETTO OIL

Saw palmetto oil comes from the berries of the *Serenoa repens* plant, a type of small palm tree found in the southeastern United States. It is widely used in herbal medicine, especially for promoting hair growth, improving prostate health, and balancing hormones. The oil contains beneficial natural compounds like fatty acids, plant-based sterols, and antioxidants that contribute to its effects.

One of its main uses is in treating benign prostatic hyperplasia (BPH), a condition where the prostate gland becomes enlarged, causing discomfort in older men. Saw palmetto oil works by blocking an enzyme called 5-alpha reductase, which converts testosterone into dihydrotestosterone (DHT)—a hormone linked to both prostate enlargement and hair loss. Because of this, it is also commonly used in hair care products to help reduce hair thinning and baldness.(8)

Saw palmetto oil is available in different forms, such as liquid extracts, soft gel capsules.

Figure 3: Saw palmetto leaf and seed

Role of saw Palmetto oil in hair fall reduction:

Saw palmetto oil plays a significant role in hair regrowth, particularly for individuals dealing with hair thinning or androgenic alopecia (male and female pattern baldness).

Its primary mechanism of action lies in its ability to inhibit the enzyme 5-alpha reductase, which is responsible for converting testosterone into dihydrotestosterone (DHT). DHT is a hormone that shrinks hair follicles, leading to hair loss. By blocking DHT production, saw palmetto oil helps reduce DHT levels, preventing follicle miniaturization and promoting healthier hair growth.

Topical application of saw palmetto oil, in the form of shampoos, conditioners, or scalp oils, can directly benefit the scalp by improving blood circulation, ensuring that hair follicles receive sufficient nutrients and oxygen.(9)

Mechanism of saw palmetto oil:

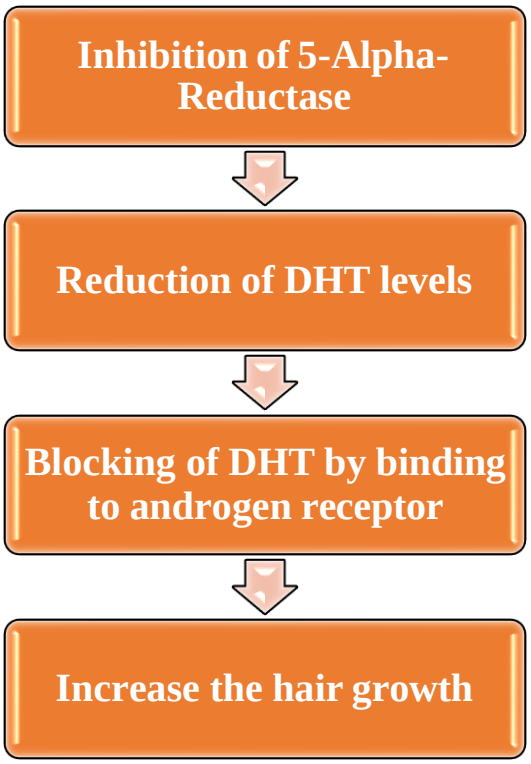


Figure 4: Mechanism of saw palmetto in hair growth

Synergistic activity of Minoxidil and Saw palmetto oil:

Combining minoxidil and saw palmetto oil creates a synergistic approach to hair regrowth by targeting both circulation and hormone regulation. Minoxidil works as a vasodilator, increasing blood flow and nutrient delivery to hair follicles, stimulating growth and extending the anagen phase. Saw palmetto complements this by potentially blocking DHT, the hormone responsible for follicle shrinkage in androgenetic alopecia. Together, they promote stronger, thicker hair growth, with minoxidil actively encouraging new hair while saw palmetto helps maintain follicle health by reducing DHT-related damage, making this combination a promising choice for hair restoration(8)

Literature Review

LITERATURE REVIEW

Umar et al. [2024] Formulated cubosomal drug delivery systems for enhancing the therapeutic efficacy of cisplatin in lung cancer treatment using glyceryl monooleate based cubosomes by top-down approach and optimized the formulation to achieve a particle size of 168.25 ± 5.73 nm, a zeta potential of -9.56 ± 1.33 mV, and an encapsulation efficiency of 60.64 ± 0.11%. In vitro drug release studies at pH 7.4 showed a sustained release profile, with cisplatin-loaded cubosomes releasing 94.5% of the drug over 30 hours, compared to the rapid 99% release from a pure cisplatin solution within 1.5 hours. Cytotoxicity assays using the NCI-H226 human lung carcinoma cell line revealed that cisplatin-loaded cubosomes exhibited comparable cytotoxicity to free cisplatin, indicating that the encapsulation did not hinder its anti-cancer activity. Meanwhile, blank cubosomes showed no toxicity, highlighting the biocompatibility of the carrier system. The study concludes that cubosomes could serve as a promising nanocarrier for controlled and sustained cisplatin delivery.

Xing et al. [2024] Combined nitric oxide (NO) with minoxidil (Mi) using hyaluronic acid liposomes (HL). Researchers formulated a transdermal complex (HL@Mi/NONOate) designed to improve drug penetration, prolong retention in the scalp, and enhance vasodilation. The effectiveness of this system was evaluated through in vitro experiments, animal studies, and histological analysis of treated hair follicles. Results showed that the NO-Mi combination significantly increased blood flow, improved follicular absorption, and stimulated hair regrowth more effectively than traditional minoxidil formulations.

Zaki et al. [2023] Developed and optimized cubosomal formulations of metformin to improve its anticancer efficacy against breast and colon cancer cells. Utilizing a Box-Behnken design via Design-Expert® software, the researchers examined the effects of varying concentrations of glyceryl monooleate (GMO), Pluronic F-127 (PF127), and Tween 80 on key formulation parameters: entrapment efficiency (EE%), vesicle size (VS), and zeta potential (ZP). The optimal formulation comprised 4.36% GMO, 5% PF127, and a negligible amount of Tween 80, achieving an EE% of 78.06%, VS of 307.27 nm, and ZP of -26.83 mV. Validation studies confirmed the accuracy of these predictions, with less than 5% variance observed.

Characterization techniques, including X-ray diffraction (XRD) and transmission electron microscopy (TEM), indicated successful encapsulation of metformin within spherical, non-aggregated cubosomal vesicles. In-vitro release studies demonstrated a sustained drug release profile, and cytotoxicity assays on MDA-MB-231 and LOVO cell lines revealed enhanced anticancer activity of the metformin-loaded cubosomes compared to free metformin. These findings suggest that cubosomal delivery systems could potentiate the anticancer effects of metformin against breast and colon cancers.

Zaker, Taymouri, and Mostafavi [2023] Formulated of cubosomal nanoparticles to enhance the delivery and palatability of azithromycin (AZ), a widely used macrolide antibiotic. Using the film hydration method, AZ-loaded cubosomes were formulated and optimized with Design Expert software, focusing on particle size, polydispersity index (PDI), and encapsulation efficiency. Characterization studies revealed spherical cubosomes with sizes ranging from 166 to 272 nm, a PDI between 0.17 and 0.33, and a high encapsulation efficiency of 80% to 92%. The antimicrobial assessment via the disc diffusion method showed that the encapsulated AZ retained its efficacy, while a taste-masking study conducted with human volunteers demonstrated a significant improvement in palatability. The study concludes that cubosomes provide an effective strategy for taste masking without compromising therapeutic efficiency.

Prabahar et al. [2023] Investigated a novel transdermal delivery system for β-sitosterol (β-ST), a natural compound with potential hair growth-promoting properties. The study aimed to enhance β-ST's skin permeation and therapeutic efficacy by formulating it into cubosomes (CUBs) and integrating these with dissolving microneedles (MN_D). They prepared CUBs using glyceryl monooleate as the lipid polymer and fabricated MN_D with hyaluronic acid and a PVP-K90 matrix. They conducted ex vivo skin permeation studies and in vivo hair growth efficacy tests. The results demonstrated that CUBs- MN_D exhibited superior β-ST permeation compared to CUBs alone, and significant hair growth was observed in the CUBs-MN_D-treated group.

Makhlouf and Elnawawy [2023] Formulated Minoxidil cubosomes (MXD-CUB) by melt dispersion emulsification technique according to full 2^3 factorial design. The optimized formula was investigated by transmission electron microscopy, X-ray diffractometry and in vitro release test. In vivo study included Draize test, histopathological examination, hair

regrowth efficacy and confocal laser scanning microscopy (CLSM). Particle size, zeta potential and polydispersity index of the optimal MXD-CUB were measured to be 131.10 ± 1.41 nm, -23.5 ± 0.42 mV and 0.185 ± 0.0, respectively, and its entrapment efficiency was 80.4 ± 4.04 %. Draize test and histopathological testing proved safety and tolerability of MXD-CUB. In vivo hair regrowth study revealed greater hair growth boosting effect of the prepared cubosomes compared to minoxidil solution.

Sudeep et al. [2023] Studied the effectiveness of phytosterol enriched saw palmetto oil in a 16-week randomized, placebo-controlled study, the efficacy and safety of VISPO™, a standardized saw palmetto oil containing 2-3% β-sitosterol, were evaluated in subjects with mild-to-moderate androgenetic alopecia (AGA). Eighty healthy male and female participants aged 18-50 were randomly assigned to receive either 400 mg oral capsules of VISPO™, a topical formulation containing 20% VISPO™, or their respective placebos once daily. The primary outcomes assessed were hair count (via hair comb and hair pull tests) and self-perceived efficacy, supplemented by global photographic assessments and phototrichogram analyses measuring hair density, thickness, and anagen/telogen ratio. At the study's conclusion, both oral and topical VISPO™ formulations significantly reduced hair fall by up to 29% and 22.19%, respectively ($p<0.001$ and $p<0.01$). Hair density increased by 5.17% in the oral group and 7.61% in the topical group ($p<0.001$). Additionally, oral administration of VISPO™ led to a notable reduction in serum dihydrotestosterone (DHT) levels compared to placebo ($p<0.001$). No serious adverse effects were reported, suggesting that VISPO™ is a safe and effective treatment for reducing hair fall and promoting hair regrowth in individuals with AGA.

Victorelli et al. [2022] Developed a vaginal drug delivery system using cubosomes to enhance the bioavailability and local absorption of curcumin, a lipophilic compound with anticancer properties. The researchers formulated mucoadhesive cubosomes using glyceryl monooleate and incorporated curcumin to assess its efficacy against cervical cancer. Characterization techniques such as small-angle X-ray scattering (SAXS), cryo-transmission electron microscopy (cryo-TEM), and dynamic light scattering (DLS) confirmed the structural integrity and appropriate size distribution of the cubosomes. Ex vivo permeation studies demonstrated that curcumin released from the cubosomes was effectively retained in the vaginal mucosa. In vitro cytotoxicity assays showed enhanced cytotoxic effects of curcumin against HeLa cells when delivered via cubosomes. The curcumin-loaded cubosomes exhibited antiangiogenic effects in vivo, evaluated using the chick embryo chorioallantoic membrane (CAM) model.

Oaku et al. [2022] Developed a 5% minoxidil nanoparticle (MXD-NP) formulation using a bead mill method to improve hair growth efficacy. When applied to C57BL/6 mice, MXD-NPs demonstrated superior hair growth compared to a commercially available minoxidil solution (CA-MXD). Notably, the MXD-NPs achieved a 7.4-fold increase in minoxidil concentration within the hair bulge region, a critical area for hair follicle epithelial stem cells (HFSCs). This targeted delivery led to enhanced activation of HFSCs, suggesting that MXD-NPs more effectively accumulate minoxidil in upper hair follicles, thereby promoting hair growth.

Oliveira et al. [2022] Reviewed cubosomes, focusing on their unique structural properties, preparation methods, characterization techniques, and diverse biomedical applications. Cubosomes are nanosized dispersions of lipid bicontinuous cubic phases in water, characterized by a lipidic interior and aqueous domains arranged in a cubic lattice. This unique architecture allows them to incorporate hydrophobic, hydrophilic, and amphiphilic compounds, making them versatile carriers for various bioactive molecules. The review discusses different preparation methods, such as the top-down and bottom-up approaches, and highlights the importance of selecting appropriate lipids like monoolein or phytantriol to achieve desired properties. Characterization techniques, including electron microscopy, X-ray scattering, and dynamic light scattering, are detailed to emphasize their role in understanding cubosome morphology and functionality. The article also explores the potential applications of cubosomes in the biomedical field, ranging from drug delivery systems that provide sustained release profiles to platforms for imaging and cancer therapeutics. The authors conclude that the exceptional properties of cubosomes position them as promising nanocarriers for various biomedical applications, warranting further research and development in this area.

Aim and objective

AIM:

- To formulate and evaluate cubosomes loaded with minoxidil and saw palmetto oil for preventing hair loss.

OBJECTIVE:

- To optimize the lipid, stabilizer concentration and homogenizer speed for preparing cubosomal nanoparticles using Box Behnken design.
- To characterize cubosomal nanoparticles for its particle size, PDI, Zeta potential and Entrapment efficiency.
- To evaluate the in vitro release of minoxidil from cubosomes.

Plan of work

PLAN OF WORK

Phase 1: Pre-Formulation studies

- Determination of melting point
- Determination of λ_{max} of minoxidil
- Standard curve of minoxidil

Phase 2: Formulation and characterization of cubosomes

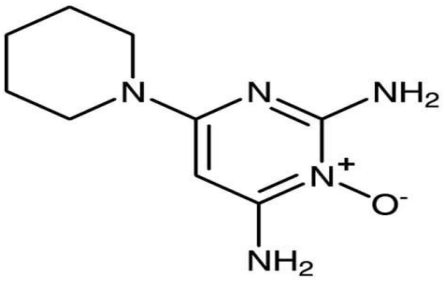
- Formulation of cubosomes by top-down method
- DOE optimization of lipid, stabilizer, oil, homogenization speed.
- Characterization-Particle size, poly dispersity index, zeta potential -by **Malvern Zetasizer.**
- Entrapment Efficiency – Ultracentrifugation method.

Phase 3: In -vitro release studies.

Drug profile

DRUG PROFILE

MINOXIDIL

Chemical structure		
Non-proprietary name	Minoxidil	
Proprietary name	Rogaine®	
IUPAC name	2,4-diamino-6-piperidinopyiridne-3-oxide	
Molecular formula	$C_9H_{15}N_5O$	
CAS number	38304-91-5	
Synonyms	Loniten, minodyl	
Appearance	White fine flavoured odourless powder	
Chemical taxonomy	Kingdom	Organic compounds
	Superclass	Organic nitrogen compounds
	Class	Amidines
	Subclass	Hyfrazine derivatives
	Molecular framework	Aromatic heteromonocyclic compounds

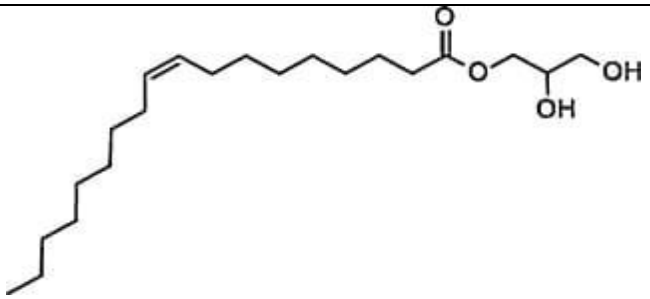
Molecular weight	209.25g/mol
Melting point	248^{o}C
BCS class	Class I
Log P value	1.24
pKa value	4.61
Mechanism of action	Vasodialation. Increase follicular growth. Reduces miniaturized hair. Anti-apoptic effect.
Uses	Hair loss treatment Hypertension treatment
Adverse effects	Scalp irritation, redness, or itching Unwanted facial hair growth (if it comes into contact with the face) Dryness or flaking of the scalp
Storage	**Temperature:** Store at room temperature (20–25°C or 68–77°F)**.** **Protection from Light:** Keep away from direct sunlight and store in a dark, cool place. **Protection from Moisture:** Avoid humid environments like bathrooms to prevent degradation.

Table 2: Drug profile of minoxidil

Excipient profile

EXCIPIENT PROFILE

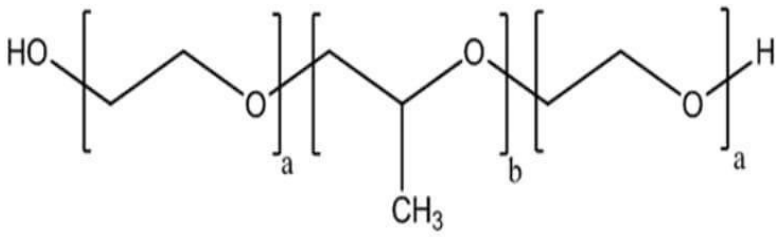
GLYCERYL MONOOLEATE

Chemical structure	
Non-proprietary name	Monegyl® - o100
Proprietary name	Glycerol mono oleate
Synonym	Monoolein, glycerol oleate. Glycerin 1-monooleate, oleylmonoglyceride
IUPAC name	2,3- dihydroxypropyl (Z)-octadec-9-enoate
Molecular formula	$C_{21}H_{40}O_4$
Molecular weight	356.5g/mol
Physical description	Pale yellow viscous oily liquid with a faint fatty odour
Odour	Sweet
Taste	Fatty taste
Nature	Non-ionic
Melting point	35^oC
Boiling point	>100^oC
Flash point	>150^oC
Density	0.942g/cm^3
Refractive index	1.4626
Solubility	It is practically insoluble in water, soluble in ether, 95% ethanol, vegetable oil, mineral oil, and chloroform.

Stability and storage condition	Store it in a cool, dry place. Use an airtight container and protect it from light
Safety	It is a relatively non-toxic and non-irritant excipient and finds use in topical and oral pharmaceutical formulations.
Application	In topical formulations, non-emulsifying grade serves dual purposes: as an emollient and as an emulsifying agent for water-in-oil emulsions.

Table 3: Excipient profile of GMO

POLOXAMER 407

Chemical structure	
Non-proprietary name	Poloxamer 407
Synonym	Pluronic F-127,Synperonic PE/F-127, Kolliphor P407, Poloxalene
IUPAC name	2-[2-(2-hydroxyethoxy)propoxy]ethanol.
Molecular formula	$HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_{54}O)_aH$
Molecular weight	9840-14600
Physicial description	Pale yellow viscous oily;liquid with a fainty fatty odour
Odour	Odourless
Taste	Tasteless
Melting point	52-57^oC
Boiling point	>149^oC
HLB value	18-23
Solubility	Freely soluble in in water, ethanol (95%) and propam-2-ol.
Storage condition	Store in a cool, dry, and well-ventilated place. Keep the container tightly closed.
Stability	Aqueous solutions of poloxamers are stable in the presence of alkalis, metal ions, and acids. But aqueous solutions promote mold growth.

Safety	Poloxamers are well-regarded in pharmaceutical formulations because they are non-toxic and non-irritant. They can be used in oral, parenteral, and topical formulations. Poloxamers are not affected by metabolic processes in the body, making them suitable for various pharmaceutical applications.
Application	Used as gelling, spreading, stabilizing and wetting agents; as a Suppository base; as a tablet coating agent; tablet excipient in different concentrations.

Table 4: Excipient profile of poloxamer 407

Materials and Equipments

MATERIALS AND EQUIPMENTS

LIST OF MATERIALS:

S.NO	INGREDIENTS	VENDOR
1	Minoxidil	Kumar organic products limited, Banglore ,India.
2	Saw palmetto oil	Veda oils, Wazirpur Industrial Area, Ashok Vihar, Delhi
3	Poloxamer 407	YARROW CHEM PRODUCTS, Mumbai, India.
4	Glyceryl monooleate	Mohini organic's Pvt Ltd, Mumbai, India

Table 5: List of materials

LIST OF EQUIPMENTS:

S.NO	EQUIPMENT	MODEL	COMPANY
1	Analytical balance	MAB 220T	WENSER
2	digital pH meter	μ pH SYSTEM 361	SYSTRONICS
3	Magnetic stirrer	1 MLH	REMI
4	Zeta sizer	NANO ZS-90	MALVERN
5	UV spectrophotometer	UV 1650PC	SHIMADZU
6	High speed homogenizer	GLH 850	OMNI INTERNATIONAL
7	Centrifuge	5810 R	EPPENDORF

Table 6: List of equipments

Methodology

METHODOLOGY

PHASE I

PRE-FORMULATION STUDIES

A) MELTING POINT:

Minoxidil's melting point was determined using capillary tube with the help of a digital melting point apparatus. After gently heating one end of a capillary tube with a Bunsen burner, a small amount of minoxidil was added to the sealed capillary tube filled with drug was kept inside the apparatus. The temperature at which it begun to melt was recorded as the melting point of the drug.

B) DETERMINATION of LAMBDA MAX:

A 10mg sample of the drug was weighed using a balance. Minoxidil was then dissolved in 10mL of phosphate buffer, resulting in a concentration of 1000mcg/mL. From this solution, 1mL was pipetted and transferred to a 10mL standard flask, which was then filled with buffer up to 20mL to achieve a concentration of 100mcg/mL. To prepare a 1mcg/mL solution, 0.1mL of the previous solution was taken and diluted to 10mL with methanol. The resulting 1mcg/mL Minoxidil solution was scanned using a double-beam ultraviolet-visible spectrophotometer in the spectrum mode from 200nm to 400nm, and the maximum absorbance at the specific wavelength was recorded.

C) STANDARD CURVE FOR MINOXIDIL:

To prepare a 1000mcg/mL concentration of Minoxidil, 10mg of Minoxidil was dissolved in 10mL of phosphate buffer. To obtain a 100mcg/mL concentration, 1mL of this solution was taken and diluted with 10mL of buffer. For a 10mcg/mL concentration, 1mL of the 100mcg/mL solution was diluted to 10mL with phosphate buffer. To achieve concentrations of 0.2, 0.4, 0.6, 0.8, and 1mcg/mL, 0.2, 0.4, 0.6, 0.8, and 1mL of the 10mcg/mL solution were each diluted to 10mL with phosphate buffer.

PHASE II

FORMULATION OF MINOXIDIL LOADED CUBOSOMES:

Minoxoidil and saw palmetto oil loaded cubosomes was prepared by top-down technique. GMO and Poloxamer 407 were melted in a water bath along with 50mg of drug and 50mg of saw palmetto oil at 70^{o}C. It was stirred in a magnetic stirrer for 15 minutes. While stirring, 4ml of water is added to the formulation dropwise using a 5mL syringe. Then the formulation is kept stationary for 48 hours to attain equilibrium. After 48 hours the formulation is reconstituted with 16mL of water and stirred in a magnetic stirrer for 30minutes.Then it is subjected to high-speed homogeniser to attain cubosomal dispersion, Then the produced cubosomes underwent further examination and testing.

Statistical design of minoxidil Loaded cubosomes:

A Box Behnken design was applied to determine the effect of different formulation variables on the particle size (nm), zeta potential, poly dispersity index. Design Expert® software (Ver. 13, Stat-Ease, Minneapolis, Minnesota, USA) was used. The formulation varied in GMO concentration between 5 and 10 (w/w %), Poloxamer concentration from 1 to 5 (w/w %) and homogenization speed from 10000 to 20000 (rpm). The amount of drug was maintained constant in all formulations at 50 mg/mL. This resulted in 17 experimental runs

Formulation variables	**Levels**	
	Low	**High**
GMO w/w%	5	10
POLOXAMER w/w%	1	5
HOMOGENIZATION SPEED (rpm)	10000	20000
Responses		
PARTICLE SIZE (nm)	Minimize	
ZETA POTENTIAL (mv)	Minimize	
PDI	Optimise	

Table 7: Formulation factors with their levels and responses

Formulation from DoE:

Run	GMO w/w%	POLOXAMER w/w%	HOMOGENIZATION SPEED (rpm)
1	5	3	10000
2	10	3	20000
3	7.5	1	20000
4	7.5	1	10000
5	10	3	10000
6	7.5	3	15000
7	10	1	15000
8	7.5	5	10000
9	7.5	3	15000
10	10	5	15000
11	7.5	3	15000
12	7.5	5	20000
13	5	1	15000
14	5	5	15000
15	7.5	3	15000
16	7.5	3	15000
17	5	3	20000

Table 8: Formulations from DoE

CHARACTERISATION OF CUBOSOMES:

A) Determination of particle size and poly dispersity index:

A ZetaSizer ZS90 (Malvern instrument, UK) was used to determine the particle size and poly dispersity index of the prepared cubosomes using dynamic light scattering technique. Measurements were performed at 90^{o}degree angle following sample diluting with distilled water.

B) Determination of zeta potential:

The zeta potential of prepared cubosomes was measured at 25^{o}C using a Malvern zeta sizer. The samples were placed in a polystyrene cuvette, and a dip cell was introduced, after which the measurements were made.

C) Entrapment efficiency:

The ultracentrifugation method was employed to determine the entrapment efficiency of prepared cubosomes. Briefly, 2ml. of the cubosome was filled in an Eppendorf tube and centrifuged at 4000 rpm for 15minutes. The clear liquid was separated and diluted, which contained the unentrapped drug. The diluted solution was analyzed using ultraviolet spectroscopy at 282 nm, and the entrapment efficiency of the cubosomes was determined using the following formula:

$$EE\% = \frac{\text{Amount of drug added} - \text{Amount of free drug}}{\text{amount of drug added in the formulation}} \times 100$$

PHASE- III

In vitro release study:

Cubosomes loaded with minoxidil and saw palmetto oil were subjected to an in-vitro drug release study by dialysis method. 5 mL of the cubosomes were enclosed in a dialysis bag and kept in a 500mL beaker filled with 200ml phosphate-buffered saline solution at pH 7.4. the temperature was carefully regulated at 37°C. At specified intervals of 5min, 10min, 15min, 30min, 1hr, 2rh, 3hr, 4hr, 5hr, 6hr, 7hr, 8hr and 24^{th} hr samples were withdrawn. The concentration of minoxidil was quantified by measuring the absorbance at 288 nm using a UV-spectrophotometer.

Results and Discussion

RESULTS AND DISCUSSION

PRE-FORMULATION STUDIES

A) Melting point:

Melting point of minoxidil was determined using capillary tube method. The melting point of minoxidil was found to be 248º C which correlates with the literature value (248ºC)

B) Determination of λ_{max} of minoxidil:

The highest concentration of 1mcg/mL was chosen to determine the lambda max of minoxidil using phosphate buffer (pH-7.4). From the UV spectrum, the absorption maximum was determined to be 282nm.

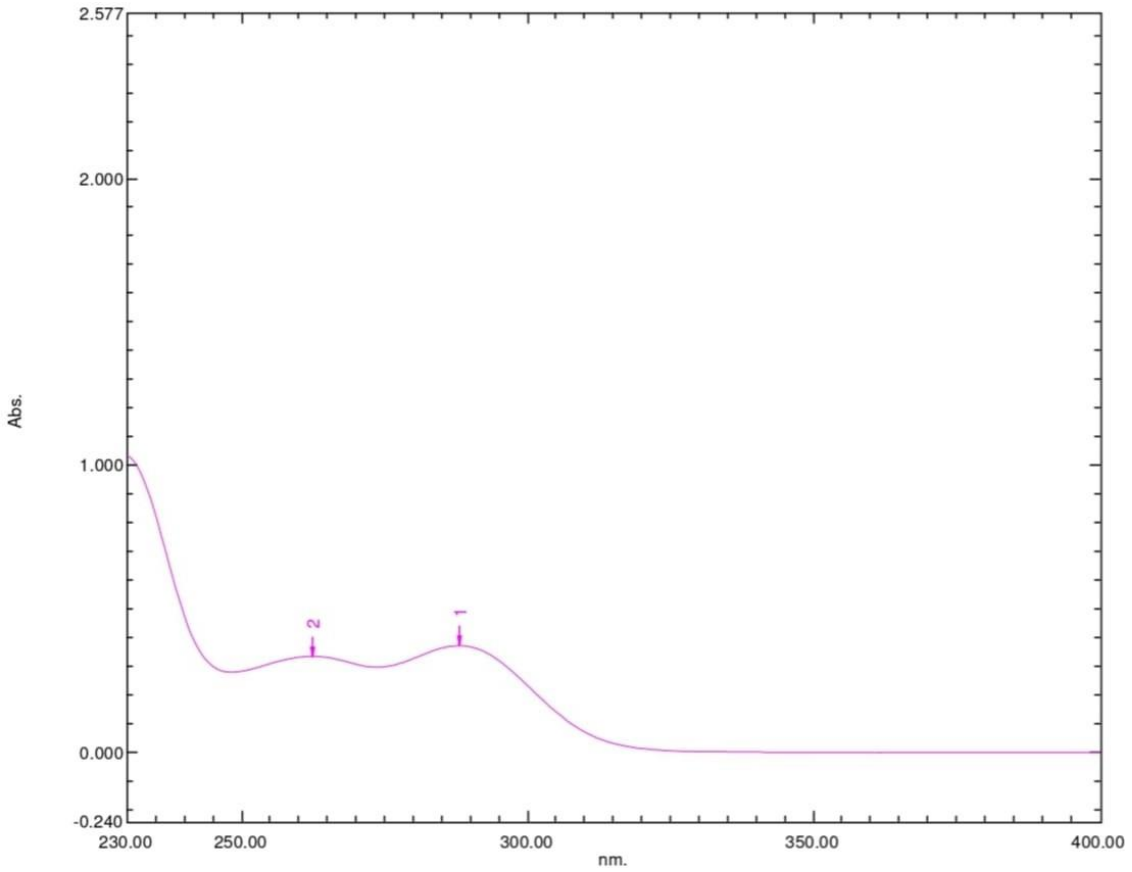


Figure 5: Lambda max of minoxidil- phosphate buffer at pH 7.4

C) Standard curve for minoxidil:

A standard graph was constructed for various concentrations of 2mcg/ml - 10mcg/mL. the absorbance was determined according to the concentration shown in table 9. The correlation coefficient was found to be r^2=0.98725.

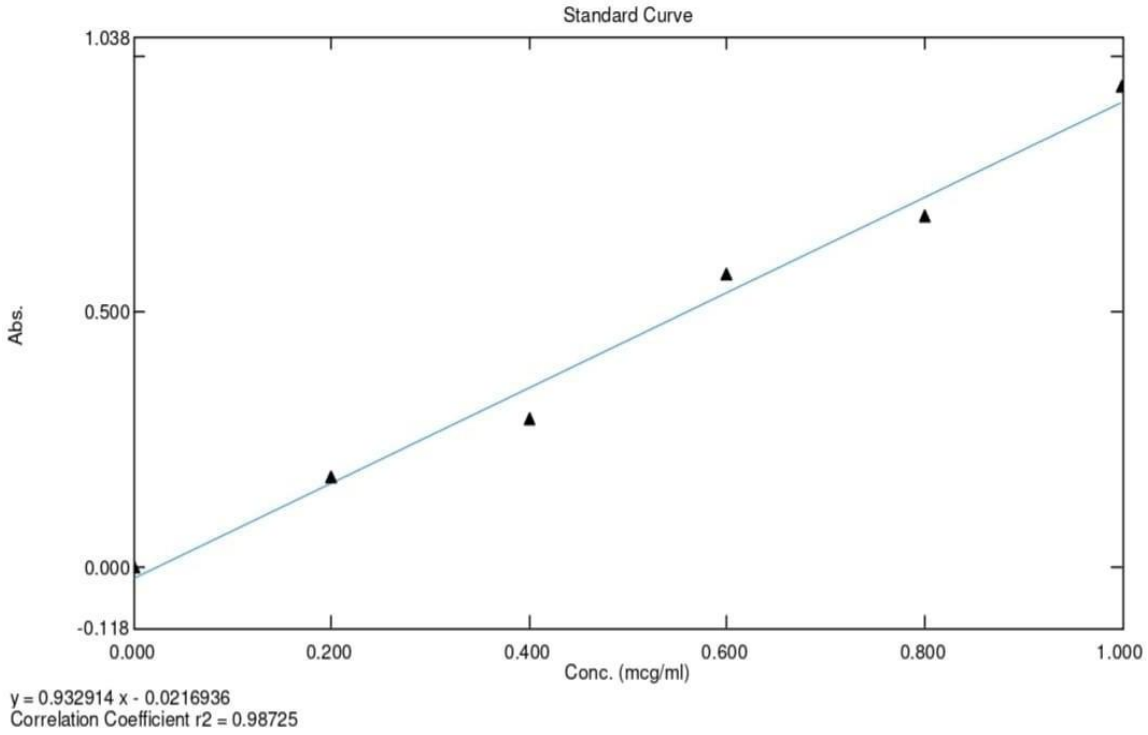


Figure 6: Standard curve data for minoxidil by UV-visible spectrophotometry using phosphate buffer pH 7.4 as solvent

S No	Concentration (mcg/ml)	Absorbance(nm)
1	2	0.175
2	4	0.289
3	6	0.573
4	8	0.689
5	10	0.942

Table 9: Standard curve data

D) Formulation of cubosomes by a top-down method:

The results of particle size, zeta potential and PD of the 17 formulations are show in the table 10.

Run	GMO	STABILISER	HOMOGENIZATION SPEED	Particle size(nm)	Poly dispersity index	Zeta potential (mV)
1	5	3	10000	145.5	0.204	-35.7
2	10	3	20000	185	0.517	-45.4
3	7.5	1	20000	157	0.264	-37.5
4	7.5	1	10000	160	0.310	-36.2
5	10	3	10000	187	0.503	-44.8
6	7.5	3	15000	162	0.330	-38.5
7	10	1	15000	180.4	0.480	-42.5
8	7.5	5	10000	175.2	0.470	-41.2
9	7.5	3	15000	155.9	0.390	-49.7
10	10	5	15000	188.5	0.542	-47.99
11	7.5	3	15000	163.4	0.380	-37.9
12	7.5	5	20000	176.5	0.420	-42.4
13	5	1	15000	147	0.195	-33.4
14	5	5	15000	157	0.201	-36.6
15	7.5	3	15000	169.5	0.410	-40.0
16	7.5	3	15000	173	0.420	-40.5
17	5	3	20000	150.1	0.240	-34.8

Table 10: Result of particle size, zeta potential and poly dispersity index of cubosomal formulations

Statistical optimization of Particle size :

Source	Sum of squares	Df	Mean square	F-value	P-value	
Model	2844.29	3	948.10	44.47	<0.0001	Significant
A-GMO	2495.71	1	2495.71	117.06	<0.0001	
B- STABILIZER	348.48	1	348.48	16.34	0.0014	
C-HOMOGENIZATION SPEED	0.1012	1	0.1012	0.0047	0.9461	
Residual	277.17	13	21.32			
Lack of fit	98.84	9	10.98	0.2463	0.9624	Not significant
Pure error	178.33	4	44.58			
Cor total	3121.46	16				

Table11: ANOVA results for particle size

The particle size value ranges from 145.5 nm to 188 nm. The linear model shows best fit. The Model F-value of 44.47 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-value of 0.25 implies the Lack of Fit is not significant relative to the pure error.

The following equation showed how the formulation factors affected the particle size

Particle size=261.476 + 60.5875 * A-27.5125 * B -4.325 * C

According to the equation, higher GMO concentration, lower poloxamer concentration and lower homogenization speed results in larger particle size.

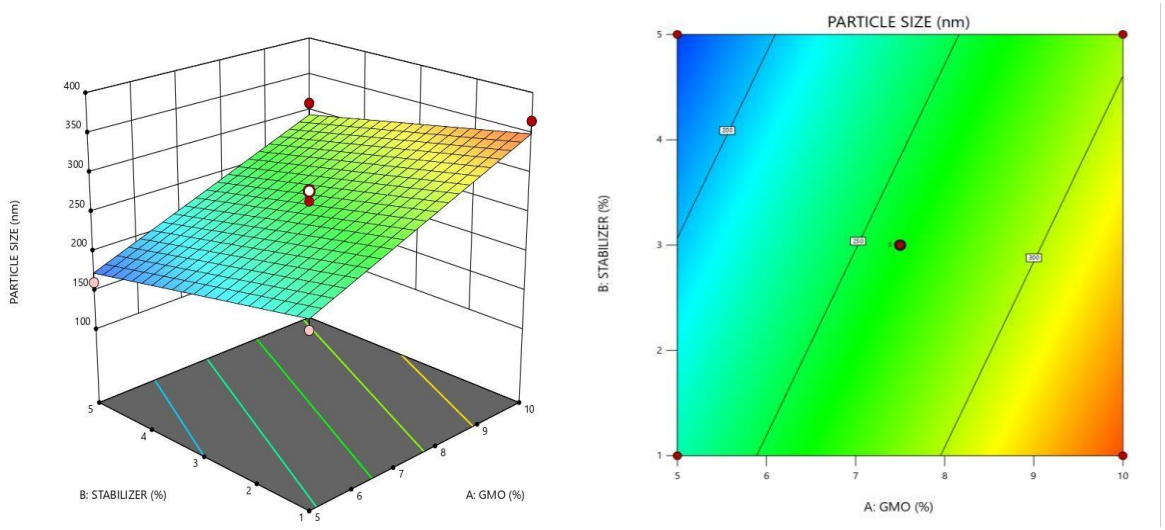


Figure 7: 3D plot and contour plot for the optimisation of particle size

Statistical optimization of poly dispersity index:

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	0.1922	3	0.0641	41.67	<0.0001	significant
A-GMO	0.1752	1	0.1752	113.95	<0.0001	
B- STABILIZER	0.0167	1	0.0167	10.89	0.0057	
C- HOMOGENIZATION SPEED.	0.0003	1	0.0003	0.1720	0.6851	
Residual	0.0200	13	0.0015			
Lack of fit	0.0151	9	0.0017	1.36	0.4089	Not significant
Pure error	0.0049	4	0.0012			
Cor total	0.2122	16				

Table 12: ANOVA result for poly dispersity index

The PDI value ranges from 0.195 to 0.542. The linear model shows the best fit. The Model F-value of 41.67 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-value of 1.36 implies the Lack of Fit is not significant relative to the pure error.

The following equation showed how the formulation factors affected the PDI

PDI = 0.5404+0.0643*A+0.0325*B-0.0060*C-0.0218*AB+0.0043*AC-0.0003*BC

According to the equation, higher GMO concentration, higher poloxamer concentration and lower homogenization speed results in higher PDI

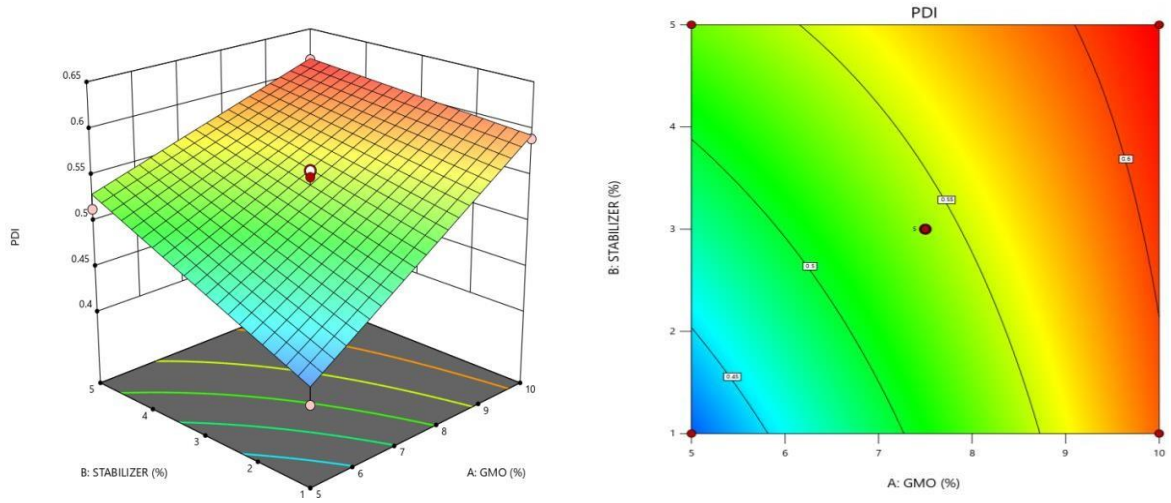


Figure 8: 3D plot and contour plot for the optimisation of PDI

Statistical optimization of Zetapotential:

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	245.71	3	81.90	10.18	0.0010	significant
A-GMO	201.90	1	201.90	25.09	0.0002	
B- STABILIZER	43.20	1	43.20	.37	0.0375	
C- HOMOGENIZATION SPEED.	0.6050	1	0.6050	0.0752	0.7882	
Residual	104.60	13	8.05			
Lack of fit	12.31	9	1.37	0.0593	0.9997	Not significant
Pure error	92.29	4	23.07			
Cor total	350.31	16				

Table13: ANOVA result for zeta potential

The zeta potential value ranges from -33.4mV to -47.99mV. The linear model shows the best fit. The model F-value of 10.18 implies the model is significant. There is only a 0.10% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-value of 0.06 implies the Lack of Fit is not significant relative to the pure error.

The following equation showed how the formulation factors affected the zeta potential

ZETA POTENTIAL = - 22.65 + 0.02625A – 3.04B – 0.05750C

According to the equation, Higher GMO concentration, higher poloxamer concentration and lower homogenization speed results in higher PDI

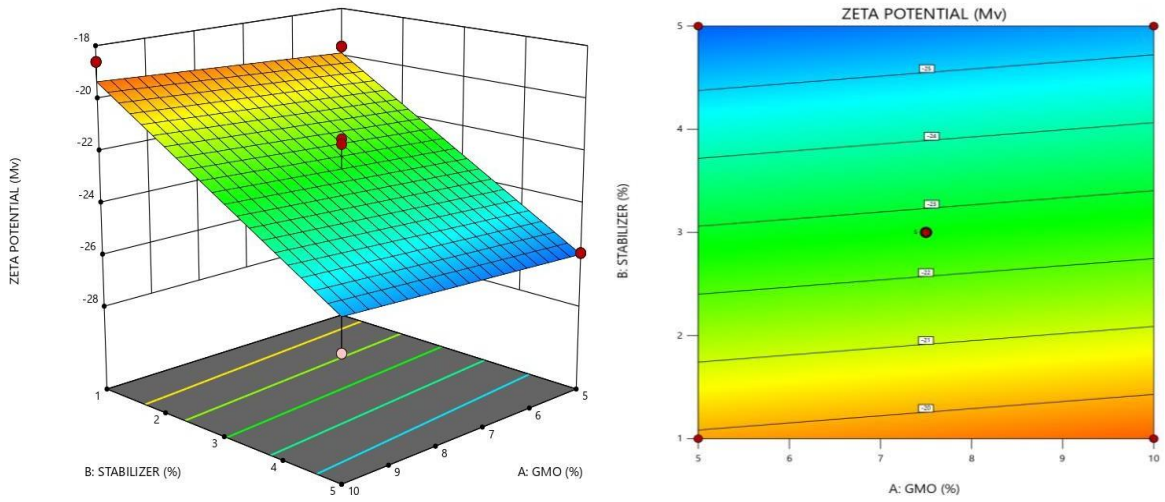


Figure 9: 3D plot and contour plot for the optimisation of zeta potential

Fit statistics:

Responses	R^2	Adjusted R^2	Predicted R^2	Adequate precession
Particle size	0.7546	0.6980	0.5533	12.1768
PDI	0.9653	0.9445	0.8511	24.0460
Zeta potential	0.9000	0.8769	0.8253	18.3611

Table 14: Fit statistics for formulation design

The difference between the adjusted and predicted R2 (difference was <0.2) The adequate precision > 4 , for all the responses indicating that the model was able to operate effectively within the designed space

Statistical analysis:

A numerical analysis was performed using Design Expert® software to determine the optimum cubosomal formulation, minimizing particle size, zeta potential and optimising the PDI. An ideal cubosomal formulation with a desirability of 0.814 was selected for the analysis. It was composed of 5 (w/w %) GMO, 1 (w/w %) poloxamer and 20000(rpm) homogenization speed. The expected values for particle size, zeta potential and PDI were 224.076nm, -22.447mV, and 0.412, respectively. The best formula was prepared and validated.

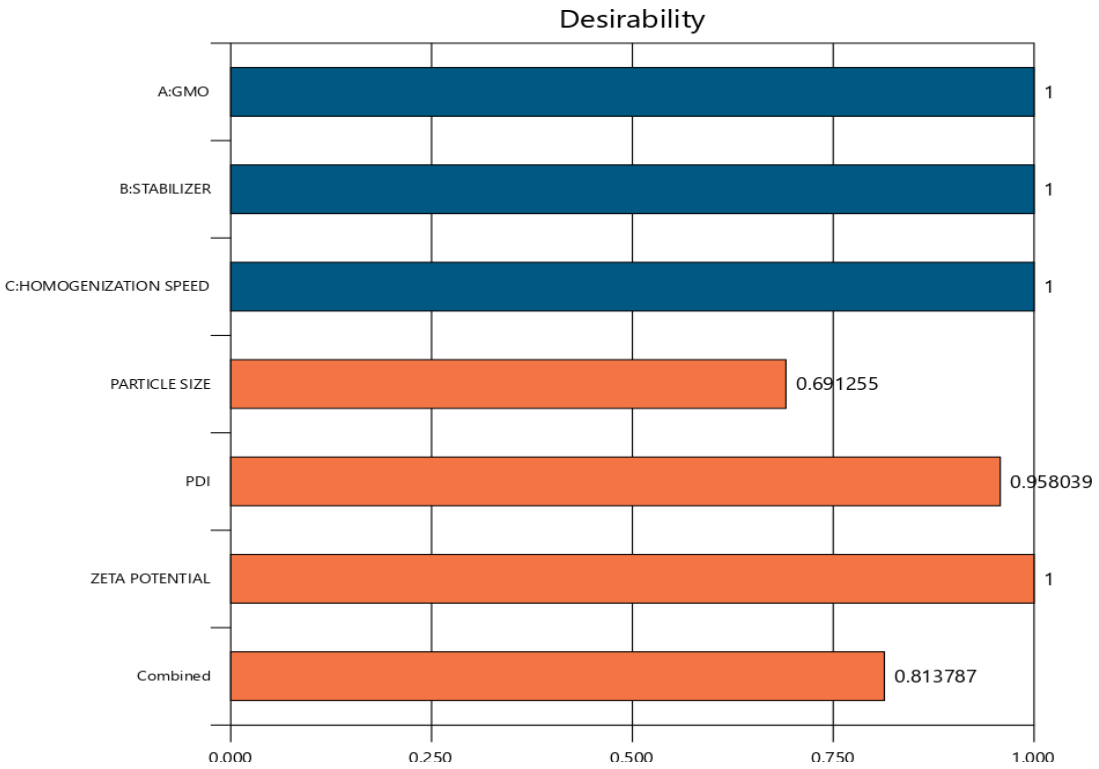


Figure 10: Statistical analysis of DoE formulations

Validation of optimal formula:

PARTICLE SIZE (nm)			ZETA POTENTIAL (mV)			POLY DISPERSITY INDEX		
Predicted	Observed	Relative error	Predicted	Observed	Relative error	Predicted	Observed	Relative error
261.4476	265.0	1.348	-22.6471	-22.5	0.653	0.540	0.568	5.19

Table 15: predicted and observed values of the optimised batch

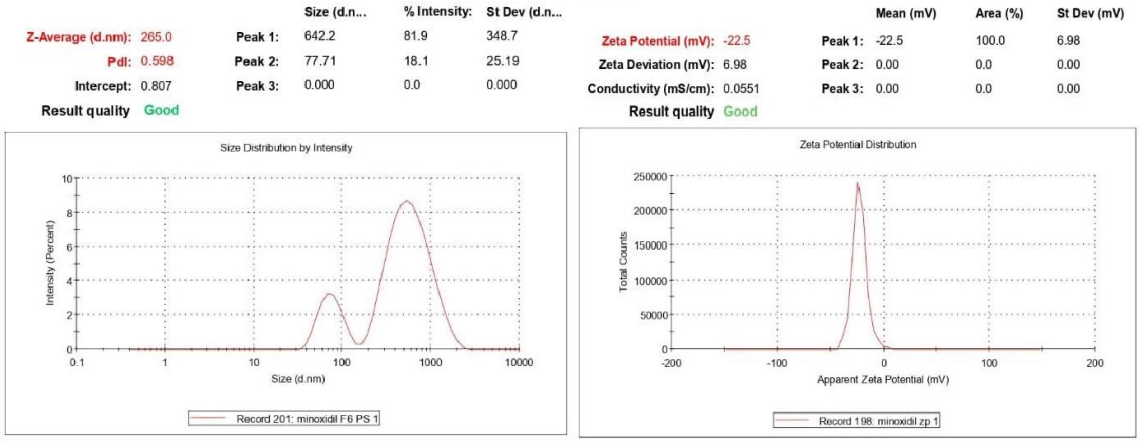


Figure 11: Results of particle size, zeta potential and PDI of optimised formulation

E) Entrapment efficiency:

The entrapment efficiency of the optimised batch was found in triplictaes and results were recorded.

Trial	% Entrapment efficiency
Trial 1	88
Trial 2	87
Trial 3	90

Table 16 : Entrapment efficiency of optimised batch

The entrapment efficiency of the triplicate samples was recorded at 88%, 87%, and 90%, which slightly exceeds the literature value of 80.04% ± 4.04%. This increase is due to the lipophilic nature of minoxidil, which has a strong affinity for GMO and poloxamer, leading to higher entrapment.

F) IN-VITRO RELEASE:

The release profile of optimised cubosomal formulation was studied for 24 hours using dialysis method in a phosphate buffer (7.4pH). The optimum cubosomal formulation revealed a sustained release profile and resulted in release of 1.780% of initial release, 5.504% in 1 hour, 28.941% in 8 hours and 42.240 % in 24 hours.

S.No	Time(min)	% Release
1	0	1.780
2	5	2.029
3	10	2.328
4	15	2.727
5	30	4.124
6	60	5.504
7	120	8.645
8	180	18.634
9	240	20.962
10	300	22.458
11	360	24.120
12	420	26.780
13	480	28.941
14	720	34.759
15	1440	42.240

Table 17: In-vitro release study data of minoxidil and saw palmetto loaded cubosomes

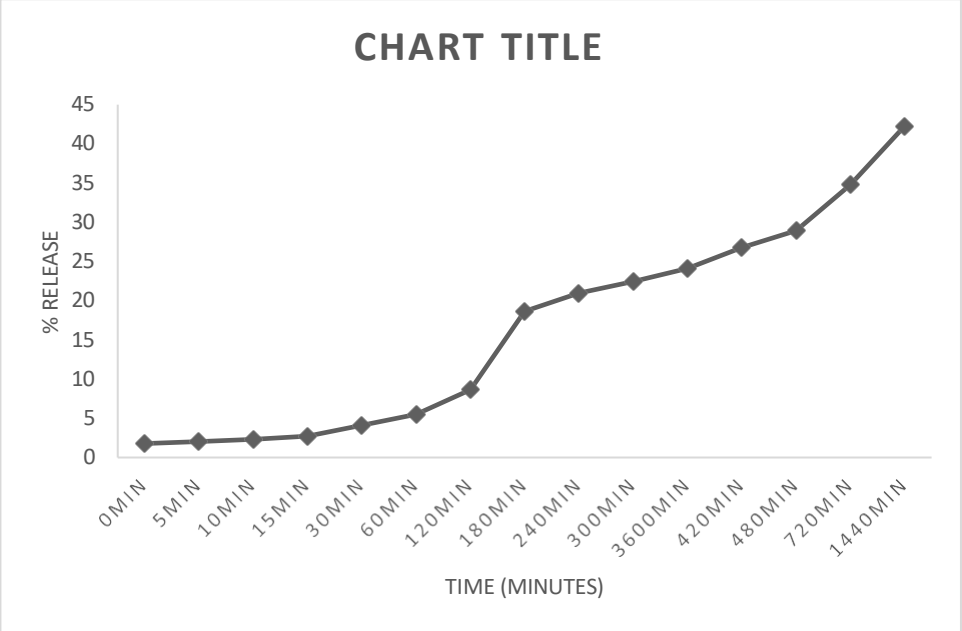


Figure 12: In- vitro release study data for minoxidil and saw palmetto loaded cubosomes.

Release kinetics:

The release data was fitted to various kinetic models like first order model (equation), zero order model (equation), Hixson-Crowell cube root (equation), Higuchi equation and Korsemeyer-Peppas equation. The mathematical kinetic modelling was performed using DD Solver to identify the pattern of drug release from cubosomes. Based on the analysis: The Higuchi model best describes the release kinetics, indicating that drug release from cubosomes follows Fickian diffusion. The n value (~0.5) from Korsmeyer-Peppas confirms diffusion-dominated release, with minimal erosion effects.

Conclusion

CONCLUSION

To conclude, the research findings showcase the successful development and characterization of a cubosome loaded with minoxidil and saw palmetto oil for preventing hair fall, through pre-formulation studies, essential properties were studies. By employing top-down approach, Box Behnken design optimized the key formulation parameters, which led to cubosomes with the desirable particle size, PDI and zeta potential. The entrapment efficiency was studied by ultracentrifugation method, the sustained release was demonstrated by in-vitro release studies. The outcome of the result provides strong basis that minoxidil and saw palmetto oil can be loaded into cubosomes successfully and could enhance the bioavailability through sustained release, offering a promising approach for prevention of hair loss.

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