Formulation Development and In-Vitro Evaluation of Glycerosomes Containing Mionoxidil for Topical Application

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ABSTRACT

Background and Objective: Novel vesicular systems provide an alternative to improve drug delivery into and through the skin. Glycerosomal vesicles have shown their ability to increase dermal drug delivery. More over, glycerosomes offer a promising avenue to fulfill the need for a transdermal drug delivery system, can act as drug containing reservoir and the modification of the vesicular compositions or surface properties can adjust the drug release rate and the affinity for the target site. Androgenetic alopecia is the most common form of alopecia in men and women. Topical minoxidil (ROGAINE 2%) became first FDA-approved medication for stimulating hair growth. In the present study an attempt has been made to improve the skin penetration of minoxidil by encapsulating it in Glycerosomes.

Method: Minoxidil glycerosomes were prepared using soya lecithin, cholesterol with different concentration of glycerol (20%, 30% and 40%) by lipid thin film hydration. Design expert 13 version has been used for the optimization of formulation. Nine formulations of minoxidil loaded glycerosomes were prepared based on 3^2 factorial designs. The prepared systems were characterized for size, zeta potential, shape, entrapment efficiency and *in vitro* drug release.

Results: Among the nine formulations F1 was selected as optimized formulation. Glycerosomes showed spherical morphology, smooth, vesicular in nature morphologically same without clusters and size range was found to be 120.63 and 186.4 nm. The entrapment efficiency was found in the range of 70.47 to 89.58% and drug release extended upto 13 hours. The kinetics of *in vitro* drug release of glycerosomes follow zero order and non- fickian diffusion mechanism.

Interpretation and conclusion: Minoxidil glycerosomes containing 40% of glycerol were found to be the effective formulation on the basis of optimization using DOE among all the other formulations and were incorporated into HPMC K100 M gel. The formulated gel was evaluated for appearance, pH, drug content, viscosity and *in vitro* drug release. The study has demonstrated the potential of minoxidil glycerosomes incorporated in gel, for the enhancement of topical absorption, which will be beneficial for topical application in androgenetic alopecia.

Key Words: Glycerosomes; Minoxidil; HPMC K100 M gel; *In vitro* drug release.

INTRODUCTION

Due to its low cost of therapy and ease of administration, the topical route is the most traditional and convenient way to administer therapeutic agents. It is also the most patient-friendly as it leads to the highest level of compliance with the therapy.

Recently, Manca et al addressed a new approach to enhance the permeability of liposomes by adding a high concentration of glycerol (10%–30%, v/v) and proposed the novel vesicular preparations as “glycerosomes”. Glycerosomes, are the modified liposomes containing a glycerol (10-50%, v/v) and phospholipid of higher percentage. Specifically, the present invention produces glycerosomes without alcohol in order to avoid

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ethanol's irritating properties when applied to the skin. A higher glycerol (>20%) concentration improve the rheological properties (e.g. Viscosity) and, consequently, give a positive contribute to the stabilization of the glycerosome vesicular system, thus glycerosomes are harmless and fully accepted compound for topical applications. It has been well documented that glycerosomes are highly flexible and deformable due to their high concentration of glycerol(1).

A glycerol concentration lower than 20% hamper the assembling of vesicles at room temperature (namely at 25°C.) when phospholipids with high transition temperatures (e.g. 70-80°C.) were used for preparing glycerosomes. Vesicles prepared with a low glycerol concentration (10-15%) display a reduced flexibility and possibly a reduced skin permeation capacity(2).

Glycerosomes improve penetration of drug in stratum corneum and deliver it to the inner layer of skin by acting as edge activator and penetration enhancer(3).

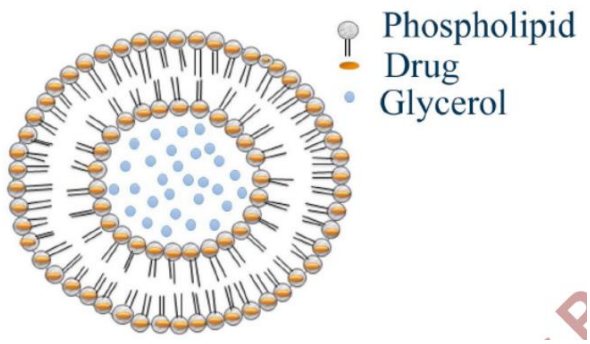

Figure 1: Glycerosome Adapted from (4).

Pharmaceutical compositions for topical application, may take a variety of forms including, for example, Solutions, gels and Suspensions. An improved absorption may be achieved when the topical compositions are in the form of a solution or gel, i.e., where the active ingredient, like, minoxidil, is dissolved in the carrier Solution, in contrast to topical compositions which are in the form of Suspensions, i.e., where the active ingredient is merely suspended in the composition.

Most of the glycerosome formulations prepared were targeted for topical delivery and in particular, a cutaneous route where they have shown promising results(5), (6). These vesicles are biocompatible and due to increased glycerol concentration, they have improved spreadability and penetrability. It is therefore imperative to explore the other topical routes such as ocular, vaginal, nasal, and rectal for delivery of drugs(3).

The positive effect of minoxidil on hair growth is partly attributed to its metabolite, minoxidil sulfate, and primarily due to its enzyme, sulfotransferase, which is located in hair follicles. Early in the 1970s, minoxidil was introduced as a treatment for hypertension. People who took minoxidil tablets experienced hypertrichosis, which was the regrowth of hair in male baldness victims(7).The mechanism of minoxidil on hair growth is believed to be its direct stimulation on hair follicle, which causes entry of the resting stage of the hair follicles to growing phase. Furthermore, enhanced microcirculation around the hair follicles and alteration of androgen effect on genetically programmed hair follicles are also contributed to the effect(8).

It is estimated that approximately 70% of men and 30% of women are affected by alopecia. Alopacia areata and androgenetic alopecia are two types of alopecia. Alopecia areata refers to round or oral, nonscarring areas of hair loss(9). Androgenetic alopecia refers to hair loss above the temple that forms an M shape and thinning at the top of the head(10).

Hence, in the present investigation an attempt will be made to develop topical gel of minoxidil glycerosomes, as glycerol acts as an edge activator and penetration enhancer. Minoxidil is selected as a suitable candidate for the present study to show effective treatment in androgenetic alopecia. Glycerosomes containing minoxidil can be an excellent therapy for Alopecia(11).

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MATERIALS AND METHODS

Method of Prepaparation of Glycerosomes:

Preparation Of Minoxidil Loaded Glyserosomes by Thin Film Hydration Method:

Lipid thin film hydration method was used in this study for preparation of Glycerosomes. Minoxidil was dissolved in Ethanol. In separate beaker Lecithin (30 mg/ml), and Cholesterol (2 mg/ml) were co-dissolved in a minimum amount of chloroform. Both organic solutions were mixed taken to 0.5 litre RBF and the organic solvent was evaporated until complete dry film was obtained under reduced pressure using a rotary evaporator. Then this dry film was hydrated using glycerol solution in phosphate buffer of pH 7.4 with a concentration ranging from 10 to 50% w/v(2),(12), (13). This Glycerosomal suspension was subjected to various evaluation studies.

Design of experiments

Optimization of various parameters of Glycerosomes by Full Factorial Design:

Nine formulations of minoxidil loaded glycerosomes were prepared based on 3^2 factorial designs, as summarized in Table 1. Two independent variables were selected which were Sonication time (X1), and Glycerol concentration (X2) as given in Table 2 and with respect to these two dependent variables were selected which were particle size(Y1), Entrapment efficiency (Y2) and % cumulative drug release after sixth hour(Y3) as per 3^2 factorial design (14), (6).

Table 1: Formulation of Minoxidil Glycerosomes.

Batch code	**X_1 (min)**	**X_2 (%W/V)**
F1	20	20
F2	30	20
F3	40	20
F4	20	30
F5	30	30
F6	40	30
F7	20	40
F8	30	40
F9	40	40

Table 2: Levels of factors for Optimization of Process Parameters of Glycerosomes Preparation:

Factor	**Name**	**Units**	**Low Level(-1)**	**Middle Level(0)**	**High Level(+1)**
A(X_1)	Sonication Time	mins	20	30	40
B(X_2)	Glycerol Conc	%W/V	20	30	40

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Evaluation of Minoxidil Glycerosomes:

Particle size:

The particle size of the prepared Glycerosomes was measured by dynamic light scattering particle size analyzer. The 1 ml of prepared formulation was diluted with 10 ml of distilled water and sonicated for 1 min with an bath sonicator before measurement and analysed in particle size analyser Horiba scientific(3).

Zeta potential:

Surface charges of all the Glycerosomal formulations were determined by zeta sizer. Zeta potential (Mv) of all the formulations was measured by laser doppler electrophoresis using the suspension of glycerosomes at the desired concentration(15).

Entrapment efficiency:

Entrapment efficiency was performed by indirect method.

Transfer the glycerosomal formulation equvivalent to 5mg into ultracentrifuge tubes. Centrifuge the sample at a high speed (e.g., 10,000 to 15,000 rpm) for 30 minutes. The unentrapped drug (free minoxidil) remains in the supernatant, while the vesicles (glycerosomes) forms a sediment. Carefully supernatant is collected, which contains the unentrapped drug. The sediment is kept undisturbed. The concentration of free minoxidil in the supernatant is measured using a UV-Vis spectrophotometer at 288 nm. The tests were carried out in triplicate(n=3)(16), (3).

Amount of entrapped drug was obtained by subtracting amount of un-entrapped drug from the total drug incorporated.The percentage drug entrapment was determined by using following equation:

Percentage Entrapment:$\frac{\text{Total drug} - \text{unentrapped drug}}{\text{Total drug}} \times 100$

***In-vitro* Drug Release Study:**

The release of Minoxidil from glycerosomal formulations were determined using Hi media dialysis membrane-110 bag diffusion technique. Minoxidil glycerosomal suspension equivalent to 5mg was taken in dialysis bag and the bag was placed in a beaker containing 200 ml of PBS pH 7.4, which acted as receptor compartment. The temperature of receptor medium was maintained at 37±0.5˚C and agitated using magnetic stirrer with the speed of 100 rpm. Aliquots of 5 ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analyzed using UV spectrophotometer at 288 nm. The tests were carried out in triplicate(n=3)(17).

Study of *in vitro* drug release kinetics:

The kinetics study of in vitro release of prepared formulations was evaluated model dependent methods (Higuchi, Korsmeyer-Peppas, zero order, and first order model). The data obtained after in-vitro release studies was subjected to fit in above four models and evaluation was done on the basis of value of regression coefficients(18), (19).

Numerical optimization:

Based on the criterias like, minimized paticle size, maximized entrapment efficiency and enhanced drug release the best formulation is selected by using Design Expert Software version 13 and this formulation is incorporated into the gel.

The selected best formulation is further subjected to the **Transmission Electron Microscopy.**

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Surface and shape analysis by using Transmission Electron Microscopy:

The surface characteristics of prepared vesicles was determined via Transmission Electron Microscope after the selection of optimized formulation by software DOE.

To visualize the Glycerosomal suspension, a drop of Glycerosomal solution was mounted on a clear glass stub, air dried and Gold coated (Icon, Mumbai) & recorded.

Formulation of Glycerosomally Entrapped Minoxidil Gel:

An optimized glycerosomal suspension containing Minoxidil equivalent to 5 % w/w was incorporated into gel base composed of HPMC K100 M and purified water(20), (21), (17).

Procedure:

Required quantity of HPMC K100 M, to prepare 25 ml of gel, was weighed and dissolved in 25 ml of purified water and kept for stirring until a homogeneous soft consistent gel is formed. At the end 0.02% of methyl paraben a preservative is added. Along with Glycerosomal suspension was formed (5%w/v).

Table 3: Formulation of HPMC gel loaded with Glycerosome containing Minoxidil.

SI.NO.	INGREDIENTS(Percentage)	Gel A	Gel B	Gel C
1.	HPMC K100 M (gm)	0.25	0.50	0.75
2.	Purified water(ml)	25	25	25
3.	Methyl paraben(gm)	0.02	0.02	0.02
4.	Glycerosome containing Minoxidil (equivalent to 5%)	5	5	5
5.	Gel texture	Low viscous gel	Soft gel with good texture	Hard gel

Evaluation of Glycerosomal Topical Gel:

Physical Appearance: The prepared gel was examined for clarity, colour, homogeneity and presence of foreign particles.

pH: The pH of dispersion was measured by using digital pH meter(22).

Rheological Study by Viscosity Measurement: Viscosity was determined by Brookfield programmable DV-E viscometer. In the present study, spindle no. CP 63 was selected with an optimum speed of 10 rpm was used to measure the viscosity of the preparation.

Spreadability: The most common method for measuring the spreadability is the parallel-plate method. This method is simple, economical, and time-effective. During the measurement using the parallel-plate method, 1 g of the sample prepared in 48 hrs before the test, is placed between two glass plates. A weight (50-500 g) of 100 g is placed on top for 1 minute. Then the diameter of the sample between the plates is measured.

In these cases, spreadability is determined by the formula: $\mathbf{Si} = \left(\mathbf{d2} \times \frac{\boldsymbol{\pi}}{\mathbf{4}}\right)$

Where,

Si- spreading area(mm^2) depending on mass,

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d- spreading area diameter(mm)

Content Uniformity:

The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of 1gm of gel in 10ml of Phosphate buffer pH 7.4 solution. The content was filtered through Whatmann filter paper No. 41. 1 ml of above solution was taken into 10 ml volumetric flask and volume was made up to mark with Phosphate buffer pH7.4 solution. The content of Minoxidil was determined at 288 nm against blank by using Shimadzu UV/ visible spectrophotometer. The drug content was determined from calibration curve of Minoxidil. The tests were carried out in triplicate.

In-vitro Drug Diffusion Study:

In-vitro diffusion study was carried out in a Franz diffusion cell using high media dialysis membrane-110 which is soaked overnight in pH 7.4 phosphate buffer. The membrane mounted between the donor compartment and the reservoir compartment of Franz diffusion cell containing 135 ml of pH 7.4 phosphate buffer. 1g of glycerosomal gel was placed over the dialysis membrane of donor compartment. The whole assembly was placed over magnetic stirrer at the temperature 37±0.5ºC. Care should be taken to avoid the entry of air bubbles below the dialysis membrane through out the study. Aliquots (5 ml) were withdrawn from the receptor compartment periodically and replaced with same volume of fresh buffer. The sample was analyzed by using UV-visible spectrophotometer at 288 nm. The tests were carried out in triplicate(n=3)(23), (24).

RESULTS& DISCUSSION

Prepared Minoxidil Loaded Glyserosomes by Thin Film Hydration Method:

Minoxidil loaded glycerosomes were successfully prepared by Thin film Hydration Method.

Design Of Experiment

Optimization of various parameters of Glycerosomes by Full Factorial Design:

The results obtained after implementing 3^2 Full Factorial design are summarized in Table 4.

Table No 4: Evaluated Parameters of Minoxidil Glycerosomes:

Batch code	**A: sonication time**	**B: Glycerol Conc (%W/V)**	**Y1 Particle size(nm)**	**Y2 Entrapment efficiency (%)**	**Y3 Cumulative Drug Release at 6^{th} hrs**	**Zeta potential (mV)**
F1	**-1(20)**	**+1(40)**	**120.63**	**89.58**	**55.833**	**-34.6**
F2	0(30)	+1(40)	121.08	85	56.277	-23.7
F3	+1(40)	+1(40)	170.6	83.29	57.083	-32.1
F4	-1(20)	0(30)	147.9	76.64	55.138	-25.4
F5	0(30)	0(30)	162.3	73.27	55.611	-33.4
F6	+1(40)	0(30)	178.9	70.47	57.083	-23.8
F7	-1(20)	-1(20)	153.1	77.76	42.22	-24.5
F8	0(30)	-1(20)	173.3	78.92	52.22	-25.5
F9	+1(40)	-1(20)	186.4	80.41	46.52	-22.3

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Response 1: Particle size

Linear Equation in Terms of Coded Factors

Particle size = +157.21+19.05*A-16.63*B

A* = Sonication time

B* = Glycerol

The particle size of all the formulation was found in the ranging from 120nm - 186nm.

According to coded factor equation of particle size, notable decrease in particle size is observed with the increase in glycerol concentration in glycerosome formulations. Specifically, as the glycerol concentration was elevated from 20% to 40%, the particle size consistently decreased. Higher concentrations of glycerol may enhance the hydration of the lipid molecules, leading to tighter packing within the vesicles. This increased packing efficiency reduces the overall vesicle size. Additionally, glycerol's ability to reduce surface tension at the lipid-water interface might further contribute to the formation of smaller, more stable vesicles(26), (14), (27).

Response 2: %Entrapment efficiency

Two factor interaction equation in Terms of Coded Factors:

%EE = +73.30-1.24*A+3.07*B-1.64AB+0.2333A^2+8.64B^2

The results showed that the entrapment efficiency of minoxidil loaded glycerosomes was ranged from 70.47 – 89.58 %. The entrapment efficiency of F1 formulation is 89.58%.

According to coded factor equation of entrapment efficiency, the study revealed a decrease in the entrapment efficiency of glycerosomes as the sonication time was extended. Specifically, when sonication time was increased from 20 to 40 minutes, a significant reduction in the amount of minoxidil encapsulated within the vesicles was observed, increasing sonication time was found to decrease the entrapment efficiency of glycerosomes. This reduction is likely due to the destabilization of the lipid bilayer and increased leakage of the encapsulated drug as a result of prolonged ultrasonic energy exposure. These findings emphasize the necessity of optimizing sonication time to maintain a balance between achieving desired vesicle size and maximizing drug entrapment efficiency(28).

Response 3: *In-vitro drug release* at 6th hour

The linearEquation in Terms of Coded Factors:

***In-vitro drug release* at 6^{th} hour =** +52.91 + 0.9482*A + 4.71*B

According to coded factor equation of in-vitro drug release, the results indicate that increasing the glycerol concentration in glycerosomes significantly enhances the in vitro drug release of minoxidil. While increased glycerol concentration enhances membrane fluidity, decreased sonication time results in larger, more permeable vesicles. Together, these factors synergistically increase the rate of drug release, suggesting that an optimal balance between these parameters is necessary for achieving desired drug release characteristics(29).

Table No 5: *In vitro* Drug Release studies of Minoxidil Glycerosomes.

Time (hrs)	Cumulative Percentage Drug Release*								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	**17.471±0.566**	15.833±0.898	13.444±0.833	14.055±1.012	12.222±0.772	10.555±0.429	15.833±0.003	11.527±0.09	9.583±0.123
2	**27.805±0.955**	25.555±0.876	23.333±0.482	26.916±0.786	19.722±0.909	22.222±0.789	22.236±0.212	23.333±0.005	15.277±0.654

3	**35.694±0.594**	34.027±0.921	29.027±0.875	32.472±0.221	24.583±0.311	29.027±1.09	26.805±0.008	30.694±0.540	22.361±0.613
4	**43.388±1.540**	42.638±0.561	31.388±0.544	44.583±0.007	30.277±1.209	33.888±0.211	34.305±0.097	33.888±0.764	30.972±0.538
5	**45.972±1.084**	54.305±1.033	40.833±0.899	51.25±0.009	43.194±0.785	42.083±0.980	43.194±0.132	35.972±1.031	39.166±0.561
6	**57.083±0.476**	56.277±0.980	55.833±0.613	55.611±0.325	55.277±1.057	55.138±1.053	52.222±0.564	42.222±0.732	46.527±0.712
7	**65.361±1.130**	63.194±1.029	61.255±0.583	67.083±0.632	61.805±0.532	59.166±0.061	56.805±1.09	51.527±0.980	50.416±0.765
8	**67.638±0.361**	72.638±0.696	66.805±0.422	70.833±0.711	67.777±0.712	67.777±0.006	61.666±0.778	53.888±0.325	56.944±0.321
9	**76.138**±0.980	71.083±0.007	72.361±0.544	74.694±0.772	72.777±0.785	74.027±0.005	66.527±0.561	61.666±0.899	62.5±0.540
10	**81.527**±0.696	80.694±0.898	76.805±0.789	78.472±0.654	76.805±0.921	75.27±0.2217	74.444±0.875	67.083±0.613	66.25±0.544
11	**86.527**±0.564	83.611±0.561	81.666±1.09	81.805±0.980	78.888±0.005	77.916±1.033	76.527±0.429	72.361±0.583	71.25±0.311
12	**90.972**±0.325	84.444±0.909	82.083±0.538	83.333±0.132	80.277±1.029	78.611±0.007	79.166±0.712	73.472±0.583	72.638±0.017
13	**91.083±0.361**	84.861±0.561	85.416±0.482	78.66±0.221	81.805±0.543	79.305±1.11	65.694±0.772	74.166±0.422	73.75±0.114

***Values represented as mean ± SD (n=3)**

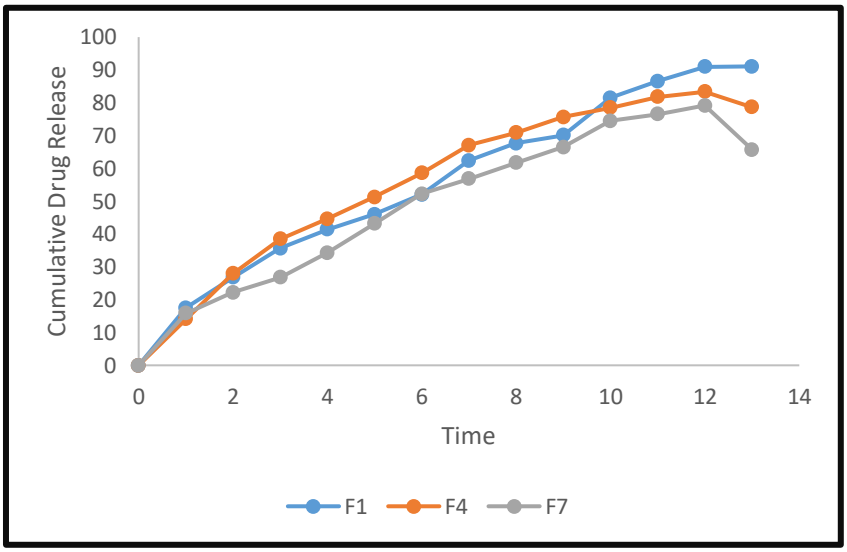

Figure No 2: *In vitro* Release Plot of Minoxidil Glycerosomes with 20 mins of sonication time having glycerol concentration 0f 40, 30 and 20% respectively.

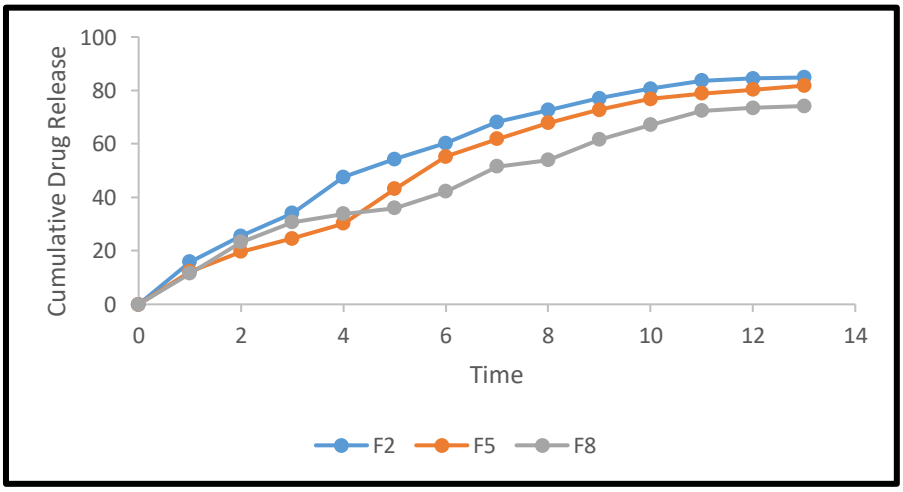

Figure No 3: *In vitro* Release Plot of Minoxidil Glycerosomes with 30 mins of sonication time having glycerol concentration 0f 40, 30 and 20% respectively.

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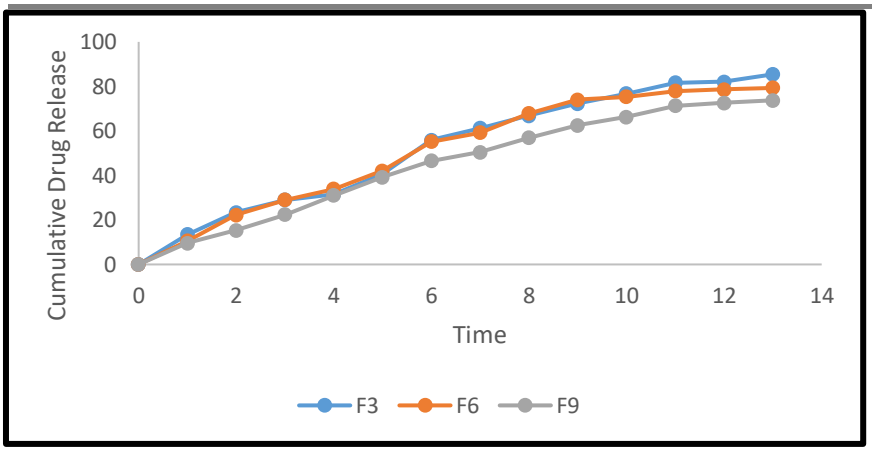

Figure No 4: *In vitro* Release Plot of Minoxidil Glycerosomes with 40 mins of sonication time having glycerol concentration 0f 40, 30 and 20% respectively.

Zeta Potential:

Zeta potential was found to be -22.3mV – 34.6mV. As the zeta potential of F1 formulation is -34.6mV.

Drug Release Kinetic Data Analysis:

Table No 6: Regression Coefficient Values of Zero order plots, First Order Plots, Higuchi Diffusion Plots and Korsmeyer-Peppas plots.

	Regression Coefficient Values			
Formulation Code	**Zero Order Plot**	**First Order Plot**	**Higuchi Model Plot**	**Peppas log Plot**
F1	**0.9815**	**0.8922**	**0.9904**	**0.9863**
F2	0.9486	0.8373	0.9859	0.9897
F3	0.9646	0.8571	0.9774	0.988
F4	0.9183	0.7962	0.9718	0.9781
F5	0.945	0.8571	0.9706	0.9783
F6	0.9396	0.8175	0.9755	0.9822
F7	0.9064	0.8538	0.9406	0.9692
F8	0.9774	0.8637	0.9814	0.9844
F9	0.9703	0.8525	0.9882	0.9888

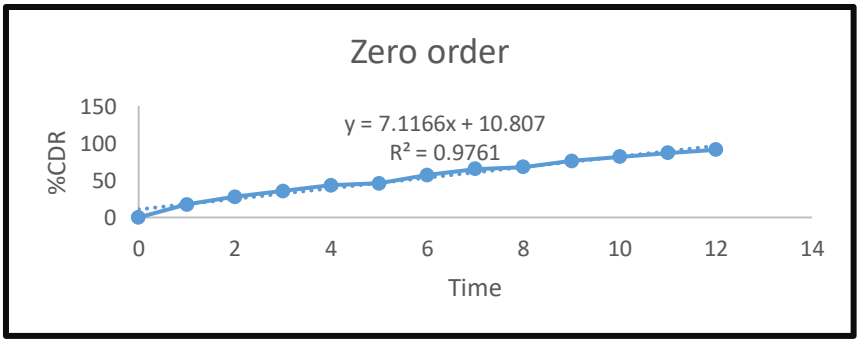

Figure No 5: Zero Order Plot of Minoxidil Glycerosomes (F1)

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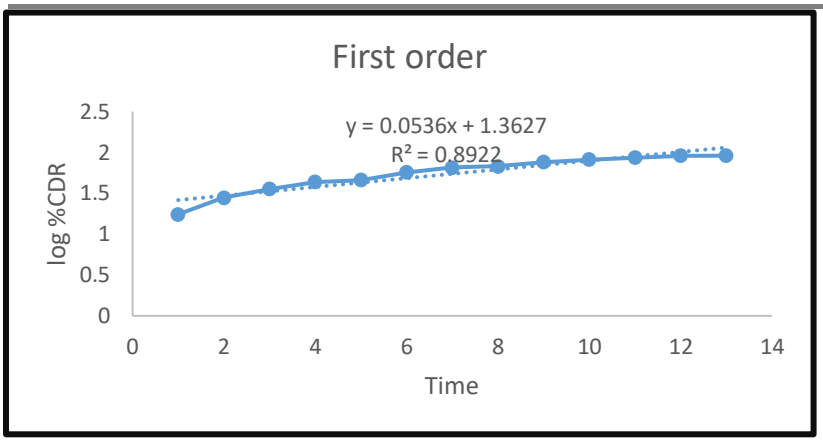

Figure No 6: First Order Plot of Minoxidil Glycerosomes (F1)

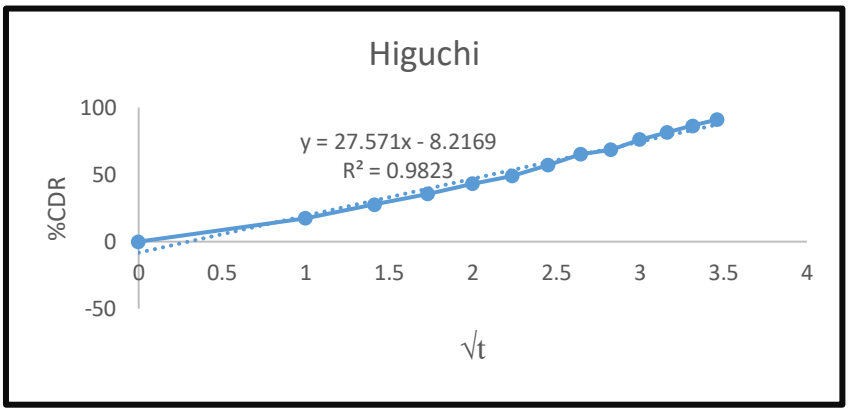

Figure No 7: Higuchi Model Plot of Minoxidil Glycerosomes (F1)

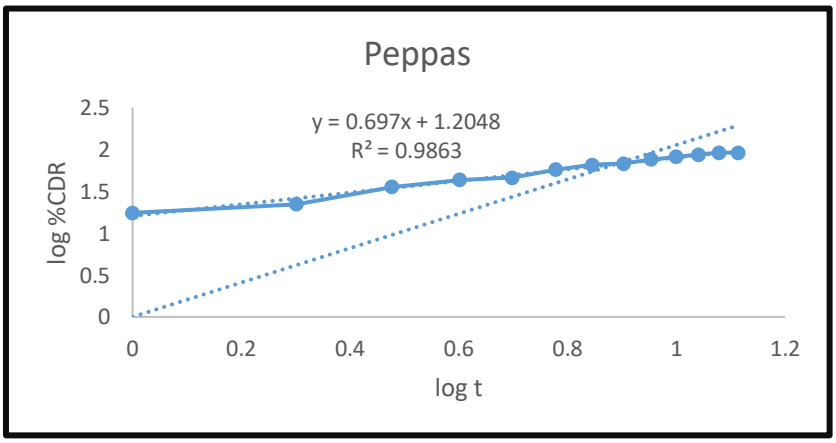

Figure No 8: Peppas log Plot of Minoxidil Glycerosomes (F1)

The interpretation of obtained data was carried out on the basis of values of the regression coefficients. The *in vitro* drug release shown that the regression coefficient values of optimized formulation (F1) for Zero order (R^2= 0.976) shown in Figure 5 , Higuchi's model (R^2= 0.982) as shown in Figure 7. Hence, the optimized formulation follows **zero order kinetics** and **non- fickian diffusion** mechanism.

Numerical optimization:

On the basis of all the parameters of evaluation tests and optimization by DOE, F1 formulation was recommended to be best formulation.

A surface characteristic of the optimized formulation (F1) was determined by TEM as shown in Fig No.9.

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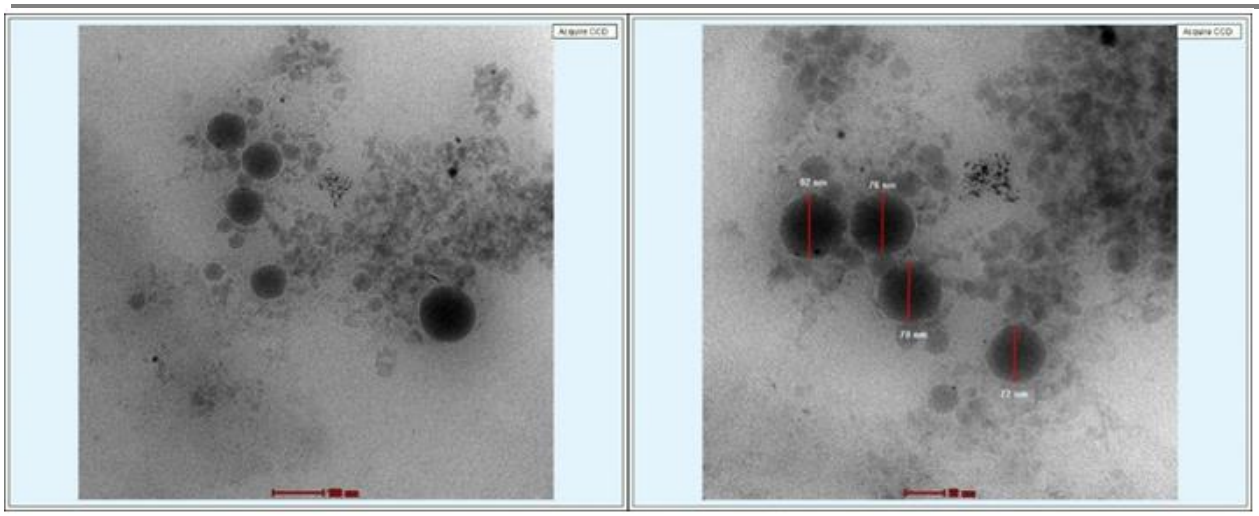

Figure No 9: TEM Images of Minoxidil glycerosomes Prepared by Thin Film Hydration Method

Surface analysis and shape by using Transmission Electron Microscopy for F1:

TEM provides detailed visualization of the size, shape, and surface characteristics of the glycerosomes

1. Vesicle Morphology (Shape):

Ideal Appearance:Spherical Vesicles: Proper lipid arrangement and hydration. This suggests effective formulation using the thin film hydration method.

2. Size: The observed vesicle sizes ranged from 120 nm, which is consistent with the particle size distribution. The vesicles appear uniform with minimal size variation.

3. Surface characteristics:

Ideal Appearance:Smooth and Well-Defined Surface: Suggests good vesicle integrity and stability.

Conclusion: These results confirm that the F1 formulation produces stable, well-formed glycerosomes suitable for drug delivery.

Preparation of HPMC K100 M gel Loaded with Minoxidil Glycerosomes:

1. 0.25% HPMC Gel: The gel prepared with 0.025% HPMC exhibited very low viscosity, resulting in a formulation that was too fluid and lacked the desired consistency. This low-viscosity gel was difficult to apply evenly and did not provide the required structure for effective topical use.

2. 0.50% HPMC Gel: At 0.050%, the gel showed a balanced viscosity, offering a soft yet stable consistency. This formulation was ideal for topical application, as it provided sufficient thickness for proper adherence to the skin without being too sticky or difficult to spread. The soft texture of the gel also made it comfortable for patient use, offering both ease of application and desired therapeutic benefits.

3. 0.75% HPMC Gel: The gel with 0.075% HPMC was highly viscous, making it too thick and more difficult to apply. While this formulation may be suitable for certain applications where a firmer gel is required, it does not meet the requirements for a soft, spreadable, and easy-to-apply topical gel.

In conclusion, the 0.050% HPMC concentration strikes an optimal balance between viscosity and ease of application. It provides a soft gel that is stable, easy to spread, and suitable for topical use, making it the preferred choice for this formulation.

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Prepared HPMC K100 M Gel composed of 0.05% of HPMC, Purified water and Methyl paraben added with Minoxidil Glycerosomes(F1).

Evaluation of Glycerosomal Gel:

Table No 7: Results of Various Evaluation Parameters of Minoxidil Glycerosomal Gel B(F1)

Sl. No.	Parameter	Result*
1	Appearance	Off-white
2	Homogeneity	Good
3	Spreadability	11.939
3	pH	6.64 ± 0.165
4	Percent drug content	91.40 ± 0.815%
5	Viscosity(cps)	3590±0.211

***Value represented as mean ± SD (n=3)**

***In-vitro* Drug Diffusion Study:**

Table No 8: Cumulative Drug Release studies of Minoxidil Glycerosomal gel

Sl.No	Time(hours)	% Drug release
1.	1	9.860±1.531
2	2	17.289±0.287
3	3	22.333±0.687
4	4	26.230±0.626
5	5	28.093±0.706
6	6	31.651±1.226
7	7	35.058±1.203
8	8	39.357±0.875
9	9	44.617±0.776
10	10	51.259±0.672
11	11	62.102±0.879
12	12	76.475±0.698

The diffusion studies focused on the optimized gel F1, which exhibited drug release percentage of76.475±0.698 %(Table 9) upto 12 hours. HPMC K100M, known for its high viscosity and matrix formation, demonstrated a slow drug release from the gel(30).

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CONCLUSION

In this study, a new vesicular carrier containing different concentration of glycerol, has been developed and characterized, which exhibit many features for topical application of cosmetic and pharmaceutical products, such as, the controlled release and targeting of drugs, occlusion associated with penetration enhancement, increase of skin hydration and excellent tolerability. Glycerosomes with anti-alopecia drug was successfully developed by lipid thin film hydration method. Morphological investigations showed that all vesicles exhibit a spherical shape with absence of aggregates and a smooth surface independent of their composition. Selection of the appropriate experimental conditions result in the production of minoxidil loaded glycerosomes having particle size (120.63nm), high entrapment efficiency (89.58%) and high cumulative percent drug release (91.083 ± 0.361%) at 13th hour. The optimum formulation was incorporated into a gel formulation and performance was evaluated *in vitro.*

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REFERENCES

1. Deepika Rani CS Arvind Kumar, Vinit Kr Sharma. Formulation Development and In-vitro Evaluation of Minoxidil Bearing Glycerosomes. American Journal of Biomedical Research. 2016;4(2):27–37.
2. Rani D, Sharma V, Manchanda R, Chaurasia H. Formulation, Design and Optimization of Glycerosomes for Topical Delivery of Minoxidil. RJPT. 2021 May 26;2367–74.
3. SWARUPANJALI PADHI PG. Glycerosomes: Advanced Liposomal Drug Delivery System. Indian J Pharm Sci. 2020;82(3):385–97.
4. Sharma D, Rani A, Singh VD, Shah P, Sharma S, Kumar S. Glycerosomes: Novel Nano-Vesicles for Efficient Delivery of Therapeutics. RADDF. 2023 Sep;17(3):173–82.
5. Sfouq Aleanizy F, Yahya Alqahtani F, Alkahtani HM, Alquadeib B, Eltayeb EK, Aldarwesh A, et al. Colored Polymeric Nanofiber Loaded with Minoxidil Sulphate as Beauty Coverage and Restoring Hair Loss. Sci Rep. 2020 Mar 5;10(1):4084.
6. Zhu C, Zhang Y, Wu T, He Z, Guo T, Feng N. Optimizing glycerosome formulations *via* an orthogonal experimental design to enhance transdermal triptolide delivery. Acta Pharmaceutica. 2022 Mar 1;72(1):135–46.
7. J.RUNDEGREN AGM. Minoxidil: mechanisms of action on hair growth. British Journal of Dermatology. 150(2004):186-194.
8. Alam MdS, Sultana N, Rashid MdA, Alhamhoom Y, Ali A, Waheed A, et al. Quality by Design-Optimized Glycerosome-Enabled Nanosunscreen Gel of Rutin Hydrate. Gels. 2023 Sep 15;9(9):752.
9. Lopedota A, Cutrignelli A, Denora N, Laquintana V, Lopalco A, Selva S, et al. New ethanol and propylene glycol free gel formulations containing a minoxidil-methyl- **β** -cyclodextrin complex as promising tools for alopecia treatment. Drug Development and Industrial Pharmacy. 2015 May 4;41(5):728–36.
10. Lizeth Martinez-Jacobo CDVV Rocío Ortiz López, Jorge Ocampo Candiani, Augusto Rojas Martínez. Genetic and molecular aspects of androgenetic alopecia. Indian Journal of Dermatology, Venereology and Leprology. 2018;84(3).
11. Khan MZU, Khan SA, Ubaid M, Shah A, Kousar R, Murtaza G. Finasteride Topical Delivery Systems for Androgenetic Alopecia. CDD. 2018 Aug 16;15(8):1100–11.
12. Deepika Rani CS Arvind Kumar, Vinit Kr Sharma. Formulation Development and In-vitro Evaluation of Minoxidil Bearing Glycerosomes. American Journal of Biomedical Research. 2016;4(2):27–37.
13. Manca ML, Zaru M, Manconi M, Lai F, Valenti D, Sinico C, et al. Glycerosomes: A new tool for effective dermal and transdermal drug delivery. International Journal of Pharmaceutics. 2013 Oct;455(1–2):66–74.

14. Zaki RM, Alfadhel MM, Alossaimi MA, Elsawaf LA, Devanathadesikan Seshadri V, Almurshedi AS, et al. Central Composite Optimization of Glycerosomes for the Enhanced Oral Bioavailability and Brain Delivery of Quetiapine Fumarate. Pharmaceuticals. 2022 Jul 29;15(8):940.
15. Uprit S, Kumar Sahu R, Roy A, Pare A. Preparation and characterization of minoxidil loaded nanostructured lipid carrier gel for effective treatment of alopecia. Saudi Pharmaceutical Journal. 2013 Oct;21(4):379–85.
16. Zhang K, Zhang Y, Li Z, Li N, Feng N. Essential oil-mediated glycerosomes increase transdermal paeoniflorin delivery: optimization, characterization, and evaluation in vitro and in vivo. IJN. 2017 May;Volume 12:3521–32.
17. Rao GV . Reddy MS Mutalik S. Preparation and evaluation of minoxidil gels for topical application in Alopecia opical application in Alopecia. Indian Journal of Pharmaceutical Sciences. 2006;68(4):432-8.
18. Costa P LJM. Modeling and comparison of dissolution profiles. European journal of pharmaceutical sciences. European journal of pharmaceutical sciences. 2001;13(2):123–33.
19. Kuksal A TA Jain NK, Jain S. Formulation and in vitro, in vivo evaluation of extended-release matrix tablet of zidovudine: influence of combination of hydrophilic and hydrophobic matrix formers. AAPS pharmscitech. 2006 Mar;7:E1-9. AAPS pharmscitech. 2006;7:E1-9.
20. G Bhagya Buela1 VVK N Delhiraj, AR VijayKumar, Y Ismail. FORMULATION AND EVALUATION OF MINOXIDIL TRANSDERMAL GEL. Eur Chem Bull. 2023;12(S3):5307–13.
21. Singh SK, Kumar P, Jindal DK, Handa V, Bilonia J. Formulation and Evaluation of Minoxidil Gel Using Acrylamide/Sodium Acryloyldimethyl taurate copolymer for Alopecia areata. IJPSDR [Internet]. 2018 Jan 15 [cited 2024 Jan 29];10(01). Available from: http://ijpsdr.com/index.php/ijpsdr/article/view/581
22. U. D. Shivhare,, k. B. Jain, v. B. Mathur, k. P. Bhusari, a. A. Roy. Formulation development and evaluation of diclofenac sodium gel using water soluble polyacrylamide polymer. Digest Journal of Nanomaterials and Biostructures. 2009;4(2):285–90.
23. Patel Rakesh P HP Baria Ashok H. Formulation and Evaluation Of Carbopol Gel Containing Liposomes Of Ketoconazole. Part-II) Available Online On International Journal of Drug Delivery Technology. 2009;1(2):01–4.
24. Gupta GD GR. Release rate of tenoxicam from acrypol gels. he Indian Pharmacist. 2005;4(35):69–76.
25. Mohammed YB, Alqahtani A, Lakshmi S, Gnanaprakash K, Kumarappan C. Studies on formulation development and evaluation of tolnaftate-loaded glycerosomes. Materials Research Innovations. 2021 Jun 7;25(4):233–42.
26. Vikash, Kumar V. Ultrasonic-assisted de-agglomeration and power draw characterization of silica nanoparticles. Ultrasonics Sonochemistry. 2020 Jul;65:105061.
27. Taladrid D, Taladrid D, MD Alemán A, Álvarez Acero I, Montero P, Gómez Guillén MC. Effect of chemical composition and sonication procedure on properties of food-grade soy lecithin liposomes with added glycerol. Food Research International. Food Research International. (100):541–50.
28. Md S, Alhakamy NA, Aldawsari HM, Husain M, Khan N, Alfaleh MA, et al. Plumbagin-Loaded Glycerosome Gel as Topical Delivery System for Skin Cancer Therapy. Polymers (Basel). 2021 Mar 17;13(6):923.
29. Naguib MJ, Salah S, Abdel Halim SA, Badr-Eldin SM. Investigating the potential of utilizing glycerosomes as a novel vesicular platform for enhancing intranasal delivery of lacidipine. International Journal of Pharmaceutics. 2020 May;582:119302.
30. Prajapati ST, Patel CG, Patel CN. Formulation and Evaluation of Transdermal Patch of Repaglinide. ISRN Pharmaceutics. 2011 Jul 20;2011:1–9.
31. Akombaetwa N, Ilangala AB, Thom L, Memvanga PB, Witika BA, Buya AB. Current Advances in Lipid Nanosystems Intended for Topical and Transdermal Drug Delivery Applications. Pharmaceutics. 2023 Feb 15;15(2):656.
32. Abdallah MH, El-Horany HES, El-Nahas HM, Ibrahim TM. Tailoring Risperidone-Loaded Glycethosomal In Situ Gels Using Box–Behnken Design for Treatment of Schizophrenia-Induced Rats via Intranasal Route. Pharmaceutics. 2023 Oct 24;15(11):2521.
33. Touitou E, Natsheh H. The Evolution of Emerging Nanovesicle Technologies for Enhanced Delivery of Molecules into and across the Skin. Pharmaceutics. 2024 Feb 13;16(2):267.