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FORMULATION, OPTIMIZATION AND EVALUATION OF GLIMEPIRIDE TRANSFERO-SOMAL GEL FOR ANTIDIABETIC ACTIVITY IN RATS

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Keywords:

Glimepiride, Carbopol 934, Thin film hydration method, Entrapment efficiency

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ABSTRACT: Transferosomes are a special liposome type consisting of phosphatidylcholine and an edge activator. Glimepiride is a second-generation sulphonylurea exerting its effects in patients with type II Diabetes. Glimepiride is hydrophobic in nature possess less solubility and permeability. The study aims to formulate, optimize and evaluate Glimepiride Transferosomal gel for antidiabetic activity in Rats. Glimepiride loaded Transferosomes were prepared using thin film hydration method and were optimized using factorial design (3^2) using Design-expert® software (Version 7.0.0, Stat-Ease Inc., Minneapolis, USA); different formulations (F1-F9) were prepared. Two different independent variables were used, which include: Amount of Soya lecithin (X1), Amount of tween 80 (X2), and the responses are Entrapment Efficiency (Y1), vesicle size (Y2). The optimized formulation of Glimepiride transferosomes was incorporated into a Carbopol 934 gel base and 1.5 % of Glimepiride transferosomal gel was prepared, which was evaluated for drug content, pH, spreadability, viscosity and *in-vitro* drug release. The prepared Glimepiride transferosomes had a maximum EE% was found to be (73.25 %), particle size was found to be (323 nm), the Spreadability value was 12.88 cm. The prepared transferosomal gel was found to be 86.02%, which represents good content uniformity. The viscosity was found to 1656±1.25 cps. The percentage drug release for Glimepiride transferosomal gel was found to be 89.05±0.25. R^2 values for the optimized formulation were highest for the Higuchi model. This indicated that the drug release from all the formulations followed diffusion controlled release mechanism. Stability studies showed that Glimepiride Transferosomal gel is more stable at 4°C when compared to room temperature.

INTRODUCTION: The skin is the vast and maximum effortlessly reachable organ of the body; it serves as a prospective path of drug direction for systemic effects. However, skin is divided into 4 layers in which the upper part of the skin, the stratum corneum, represents the most resistible blockade for the drug penetration throughout the skin, which controls the transdermal bioavailability of drugs.

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Therefore, distinctive transporters are needed to fight the natural skin barrier to administer drug molecules with discrete physicochemical effects to the systemic circulation [1, 2]. Drug and vaccine transcutaneous administration is a feasible surrogate to the oral and parenteral routes of delivery.

It is viable to avoid "first-pass" deactivation with the aid of using hepatocyte, lower the chance of abdominal digestive irritation, offer steady absorption of medicine above lengthy intervals of period and decrease the density of drugging, which boom adherence [3]. Because of its big exterior portion and effects that ease drug administration, the transcutaneous path has gained awareness over the ancient period.

However, the skin is the most fruitful blockage to drug passage, which is critical to plan the drug administration means in the most effective way possible, it involves selecting a mechanism to pass the dosage into the skin surface or Percutaneous absorption is the immersion of particles along the skin and circulation throughout the body except lungs [4]. For use on the skin, different types of drug delivery systems have evolved. Cutaneous pharmacotherapy mainly aims to administer drugs to the skin to induce local effects at the application area [5].

Skin is thought to be a highly effective shield that keeps our internal in and external out. Transitioning the skin barrier property, which includes penetration of medications, synthesized materials, and biologically active substances, is the most important prerequisite and goal for transdermal delivery. As a result, numerous approaches have been tried to improve the permeation rate of different agents. One of the approaches is to implement novel transporters effective in control delivery, drug release at a preestablished pace, and selected release. This could result in increased effectiveness, protection, and patient compliance. Microparticles, nanoparticles, liposomes, elastic liposomes, niosomes, ethosomes, and other novel release systems or transporters for efficient cutanoeus and transcutanoues administration. The best delivery methods are those that are stable, non-toxic, non-immunogenic, and cost-effective. In addition, those methods must be taken up pharmaceutically, steady, biologically suited and forbearing friendly [6-9].

Transferosomes: A transferosome is a device that allows medications to be transferred from one person to another. Administered to the desired location via skin penetration [10-12] a vesicle that is ultradeformable and has a It has an aqueous core surrounded by a complicated lipid bilayer favoured format shown in **Fig. 1**. The interdependency of local composition as well as the self-regulating and selfoptimizing characteristics of transferosomes are due to their interdependency of local composition. The bilayer's form they can pass through quickly. Different transportation hurdles and then function as a cost-effective mode of transportation medication carrier for therapeutic compounds' long-term release. The approaches resulted in two new vesicular carriers [13]: transferosomes and ethosomes, which are flexible elastic lipid-based vesicles [14]. Each transferosome's inner layer is made up of an aqueous compartment surrounded by a lipid bilayer with specific features due to the insertion of "edge activators" to the vesicular membrane. Surfactants, including sodium cholate, sodium deoxycholate, Span 80, and Tween 80 are common edge activators [15].

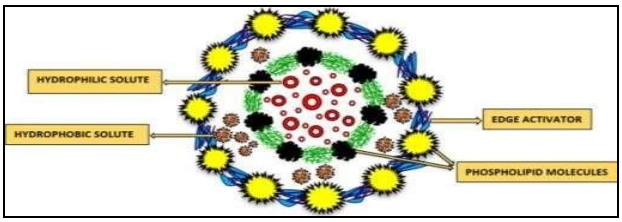

FIG. 1: TRANSFEROSOMES STRUCTURE

Diabetes Mellitus: Diabetes mellitus is a collection of metabolic illnesses marked by chronic hyperglycemia caused by insulin production, insulin action, or both. Uncontrolled diabetes may lead to stupor, coma and if not treated death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome. Type 2 diabetes is an impairment in the way the body regulates and uses sugar (glucose) as a fuel. This long-term (chronic) condition results in too much glucose circulating in the bloodstream. Eventually, high blood glucose levels can lead to disorders of the circulatory, nervous and immune systems [16].

MATERIALS AND METHODS:

Materials: Glimepiride and Soya lecithin were obtained as gift samples from Apex Laboratories limited, span 80, tween 80, sodium deoxycholate, Carbopol were purchased from S d Fine chemical Ltd, Mumbai, India. Chloroform, methanol, and triethanolamine were purchased from Otto Chemicals, Mumbai, India.

Methods:

Preparation of Glimepiride-Loaded Transferosomes using Thin Flim Hydration Method: The Glimepiride loaded transferosomes were prepared by rotary thin film hydration method [17]. An accurate amount of Soya lecithin, surfactants and Glimepiride were dissolved in a chloroform and methanol mixture (in the ratio of 2:1 v/v) in a round bottom flask as seen in **Table 1**. The organic solvent mixture was removed by using a rotary film evaporator under reduced pressure at 60 °C ± 2 °C

and 60 rpm for 15 min to get a homogeneous lipid film. The flask was kept under vacuum to remove residual solvent. The thin lipid film was hydrated with Phosphate buffer pH 6.8 above the lipid transition temperature at 60 rpm for 60 min to obtain large multilamellar vesicles (MLVs). The resulting MLVs were kept overnight at 4 °C to allow the complete hydration of the vesicles. The MLVs were then subjected to probe sonication at 4 °C for 30 min using an ultrasonic sonicator (Mangaldeep tech solutions) to get small unilamellar vesicles (SUVs) and stored at 4°C for further investigation.

Formulation Design:

TABLE 1: FORMULATION DESIGN OF GLIMEPIRIDE TRANSFEROSOMES

	Quantities in W/W % (100mg)								
Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Glimepiride (mg)	4	4	4	4	4	4	4	4	4
Soya lecithin (W/V)	20	40	60	20	40	60	20	40	60
Chloroform: methanol (V/V)	2:1	2:1	2:1	2:1	2:1	2:1	2:1	2:1	2:1
Sodium deoxycholate (w/w)	5	15	25	-	-	-	-	-	-
Span 80 (V/V)	-	-	-	5	15	25	-	-	-
Tween 80 (W/W)	-	-	-	-	-	-	5	15	25
Sodium benzoate (w/w)	1	1	1	1	1	1	1	1	1
Distilled water (v/v)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Design of Experiment: Design Expert software was employed to execute the statistical evaluation of the experimental design. The most popular response surface method was the central composite design. A 3-level design was employed in this study, requiring 9 experiments. Optimization of formulation has a great extent of influence on the composition and development method of transferosome. The 3^2 design for optimization of the transferosome was employed to study the effect of independent variables selected as soy lecithin (X1) and tween 80 (X2) on dependent variables, *i.e.*, entrapment efficiency (EE%) and size of a vesicle (nm) and respectively. The coded levels translated to the experimental units, experimental runs, and their factor combinations considered in the present study are summarized in **Table 2A and Table 2B.** The significant model was analyzed using ANOVA.

TABLE 2A: INDEPENDENT VARIABLES

Coded values Level	**Independent variables**	
	X_1, Soy lecithin	**X_2, Tween 80**
-1	30	5
0	60	15
+1	90	25

TABLE 2B: FORMULATION OF GLIMEPRIDE TRANSFEROSOMES

	Factor 1	**Factor 2**
Run	**A:Soya lecithin**	**B:Tween 80**
1	-1	-1
2	-1	1
3	1	0
4	1	-1
5	-1	0
6	1	1
7	0	1
8	0	0
9	0	-1

Characterization of Glimepiride Loaded Transferosomes:

Vesicle morphology: The morphological characteristics of the prepared vesicular formulations were examined. A drop of the vesicular formulation was homogeneously put to a clean glass slide and allowed to air dry. The sample was gold coated with a Sputter coater (JEOL, Japan) and examined under SEM with a 20kV accelerating voltage.

Entrapment Efficiency (EE): The amount of Glimepiride entrapped inside the vesicular systems was determined by ultracentrifugation technique [18]. An aliquot of the vesicular suspension was placed in the centrifuge tubes and centrifuged at 40,000 rpm for 3 hr at 4 °C. The free Glimepiride present in the supernatant was isolated from the pellet, appropriately diluted, and quantified for

Glimepiride content. To assess the total Glimepiride present in the prepared vesicular formulations, the vesicular dispersion was lysed with chloroform and subjected to sonication. The samples were analyzed under UV visible spectrophotometer.

The percentage entrapment efficiency (% EE) was determined as follows [19]:

$$EE\ \% = (T-S) / T \times 100$$

Where,

T is the total amount of Glimepiride; S is the amount of Glimepiride present in supernatant only; T-S is the amount of Glimepiride present inside the vesicles.

Zeta Potential (ZP): The ZP of the vesicular formulations was also measured by Zetasizer nano ZS using the M3PALS technique. Before analysis, samples were appropriately diluted with milli-Q water, and measurements were done out at 25 °C by determining the electrophoretic mobility.

Determination of *In-vitro* Diffusion of Glimepiride Transferosomes: A diffusion study of Transferosomes formulations was carried out using a Franz diffusion cell through a dialysis membrane. The dialysis membrane was soaked in distilled water for 24 hours. Franz diffusion cells contain two compartments upper donor and the lower receptor compartment. The receptor compartment was filled with 6.8 pH, and donor compartment contained transferosome suspension on the dialysis membrane with an exposure area of $2cm^2$ to receptor medium, and whole assembly was kept on a magnetic stirrer at 600rpm for 10 hours, and samples were withdrawn at specified time interval of 1 hr and replaced with equal volume of buffer. Samples were appropriately diluted with buffer and analyzed using UV spectrophotometer at 249nm. Steady-state Flux (Jss) was calculated from the slope of the linear part of the cumulative amount of drug permeated per unit area ($\mu g/cm^2$) against a time (h) plot.

$$\text{Permeability coefficient (Kp)} = Jss / Co$$

(Co = initial CIP concentration) [20]

Preparation of Transferosomal Gel: To make the vesicular dispersions suitable for skin application, the optimized Glimepiride -TFS formulations were incorporated into carbopol gel [21]. Briefly, carbopol 934 was dispersed in water with continuous stirring at 500 rpm to prepare 2% w/w dispersion is formed. The dispersion was mechanically stirred and then neutralized with 0.5% v/v triethanolamine solution. The dispersion was allowed to stand overnight for complete hydration and swelling. Finally, the optimized Glimepiride-TFS were added to carbopol dispersion and mixed with gentle stirring.

Characterization of Glimepiride Loaded Transferosomal Gel:

Homogeneity: Patient compliance needs to determine the homogeneity of semisolid dosage forms applied topically on the skin. This was done by pressing a small quantity of gels (GLIM Transferosomal gel) between the thumb and the index finger. The consistency was determined as homogeneous or not.

Spreadability: The Spreadability of gel formulations was determined by measuring the spreading diameter of 1g of gel between two horizontal plates (20 cm × 20 cm) [22].

$$S = M \times L / T$$

Where, S is the spreadability in g/s, M is the mass in grams L is the length of gel spread & T is the time in seconds.

pH Measurement: One gram of gel was dispersed in 20 mL of distilled water, and a digital pH meter (Systronics Digital - 335) was used to determine the pH value. The measurement was performed three times, and the mean ± SD was calculated [22].

Viscosity Measurement: The gel viscosity was measured by a Brookfield viscometer (Brookfield DVE) using spindle number S64 rotated at a speed of 12 rpm for a 10-s run time at 37°C.

Drug Content Determination: The GLIM content was measured by placing 1 g of gel onto a clean volumetric flask (100 mL) and completing the volume with 6.8 pH buffer. This was then stirred for 2 h. The solution was filtered, and samples were analyzed spectrophotometrically at 249 nm [22].

***In-vitro* Diffusion Studies:** A diffusion study of GLIM-loaded Transferosomal gel was carried out using a Franz diffusion cell through a dialysis membrane. The dialysis membrane was soaked in distilled water for 24 hours.

The receptor compartment was filled with 6.8 pH. Donor compartment contained Glimepiride Transferosomal gel on a dialysis membrane with an exposure area of $2cm^2$ to receptor medium, and the whole assembly was kept on a magnetic stirrer at 600rpm for a period of 10 hours and samples were withdrawn at a specified time interval of 1 hr and replaced with an equal volume of buffer.

Samples were appropriately diluted with buffer and analyzed using UV spectrophotometer at 249nm. Steady-state Flux (Jss) was calculated from the slope of the linear part of the cumulative amount of drug permeated per unit area ($\mu g/cm^2$) against a time (h) plot.

$$\text{Permeability coefficient (Kp)} = Jss / Co$$

(Co = initial CIP concentration.)

Release Kinetic Profile for Glimepirde Transferosomal Gel: The drug release kinetics studies were estimated to determine the type of release mechanism followed. Release kinetic study of optimized formulation was studied for different kinetic equations (zero order, first order and Higuchi & Peppas).

- **Zero-order Kinetic Model:** Cumulative %drug released versus time.
- **First-order Kinetic Model:** Log cumulative percent drug remaining versus time.
- **Higuchi's Model:** Cumulative percent drug released versus square root of time.
- **Korsmeyer-Peppas Model:** Log % CDR versus Log time.

Zero-order Kinetics: The following equation would predict Zero-order release:

$$A_t = A_0 - K_0 t$$

Where, A_t= Drug release at time't', A_0= Initial drug concentration. K_0= Zero-order rate constant (hr^{-1}).

When the data is plotted as cumulative percent drug release versus time, if the plot is linear, the data obeys zero-order kinetics and its slope equals zero-order release constant K_0.

First-Order Kinetics: The following equation could predict first-order kinetics:

$$\text{Log } C = \log C_0 - K_t / 2.303$$

Where, C= amount of drug remained at time't'. C_0= Initial amount of drug. K= First order rate constant (hr^{-1}).

The data plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follows first-order kinetics. The constant 'K_t' can be obtained by multiplying 2.303 with the slope value.

Higuchi's Model: Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation:

$$Q = [D\varepsilon / \tau (2A - \varepsilon C_S) C_S t]^{½}$$

Where, Q= amount of drug release at time't'. D = Diffusion coefficient of the drug in the matrix. A = Total amount of drug in a unit volume of the matrix. CS = Solubility of the drug in matrix. ε = Porosity of the matrix. τ = Tortuosity. t = Time (hrs at which q amount of drug is released). Above equation can be simplified as if we assume that 'D', 'C_S' and 'A' are constant. Then equation becomes:

$$Q = Kt_{1/2}$$

When the data is spited according to the equation *i.e.,* cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K' (Higuchi's 1963) [23].

Korsmeyer-Peppas Model: Korsmeyer *et al.* (1983) derived a simple relationship which described drug release from a polymeric system equation

$$Mt / M\infty = Ktn$$

Where, Mt / M∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent [24].

***In-vivo* Evaluation of Glimepiride Transfero-somal Gel:**

Animals: Albino rats of male 9-to-11-week age, weighing 180–250g were procured from Mahaveera enterprises, Hyderabad.

Animals were housed in standard laboratory conditions at 25°c with 12 hr light-dark cycle with free access to chow and water *ad libitum*. The research protocol was approved by Institutional Animal Ethical Committee (DSOP/IAEC/DEC2020/02).

Induction of Diabetes: All the rats were fasted overnight before the administration of streptozotocin. Diabetes was induced in rats by intraperitoneal injection of streptozotocin at 60 Milligram/Kilogram body weight, as shown in **Fig. 2 & 3**. Blood was taken from tale vein to examine blood glucose with a glucose monitoring device. The blood glucose was examined. The animals with more than 200 mg/dl of blood glucose levels were considered diabetic rats and were used for this study.

FIG. 2: ANIMAL HOLDER

FIG. 3: INDUCTION OF DIABETES USING STREPTOZOTOCIN

Excision Wound Model: Albino rats 180-250 gm were taken for studies; the rats were anesthetized prior to and during the infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using Anaesthetic Ether. A wound of 500 sq. mm on dorsal thoracic region was made.

- Rats were divided into three groups.
- Group I: (control group): No application of any medication.
- Group II: (standard group): Animals of this group were applied to topically marketed product (Megaheal gel).
- Group III: (test group): Animals of this group were applied topically 1.5 % Glimepiride Transfersomal gel.

The gel was applied by using a cotton bud. After the application of gel, the wounds were covered with transparent film dressing and then wrapped using a bandage to prevent the film dressing from slipping out of position.

The animals were observed for wound closure on 1st, 4, 8, 12, 14 and 16th days using a transparency sheet and a permanent marker and for a period of epithelialization.

Stability Studies: The optimized GLIM-TFS gel was subjected to stability studies in lacquered aluminium collapsible tubes stored at three different temperatures *i.e.*, 4 ± 2 °C, 25 ± 2 °C and 40 ± 2 °C for 3 months period, and evaluated for appearance, color, pH, viscosity and drug content as described earlier [21].

RESULTS AND DISCUSSION: Physicochemical properties of glimepiride **Table 3 & 4**.

Color:

TABLE 3: TABLE SHOWING THE DESCRIPTION OF GLIMEPIRIDE

Test	Description
Colour	White to yellowish white

The melting point was found to be 196.0ºC-208.0ºC by the open capillary method.

DISCUSSION: The melting point of Glimepiride was found to be 196.0ºC, which complied with standards, thus indicating the purity of obtained drug sample.

Solubility:

TABLE 4: TABLE SHOWING THE SOLUBILITY OF GLIMEPIRIDE

Solvents	Solubility (µg/ml)
Methanol	35.5 ± 0.05
Water	52.5 ± 0.07
0.1N HCl	40.5 ± 0.03
Phosphate buffer 6.8	45.5 ± 0.06

Preformulation Study:

Determination of λmax of Glimepiride: A solution of Glimipiride concentration of 10 µg/ml was scanned in the wavelength 200-400 nm range. It was observed that the API showed considerable absorbance at a wavelength of 249 nm **Fig. 4 & 5, Table 5**. The absorption spectrum was found to be sharp and maximum at wavelength of 249 nm, therefore, it was selected as the wavelength for detection in phosphate buffer 6.8.

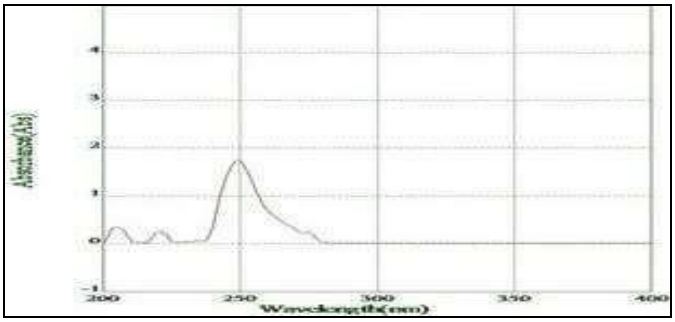

FIG. 4: ΛMAX CURVE OF GLIMEPIRIDE

TABLE 5: ABSORBANCE OF DIFFERENT CONCENTRATIONS

Concentration (µg/ml)	Absorbance
0	0.00 ± 0.00
2.5	0.145 ± 0.076
5	0.295 ± 0.012
7.5	0.467 ± 0.081
10	0.589 ± 0.105
12.5	0.751 ± 0.057
15	0.888 ± 0.089

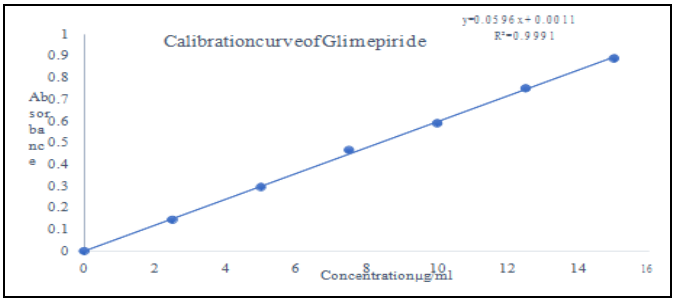

FIG. 5: CALIBRATION CURVE OF GLIMEPIRIDE

FTIR: The identity of Glimepiride obtained was confirmed by FTIR. The FTIR spectrum Glimepiride shows a characteristic sharp peak at 3367.95 cm^{-1} **Fig. 6** specifying the presence of N-H- (amine) group. The strong peak at 1669.68 cm^{-1} was assigned as the S=O (sulphur) character.

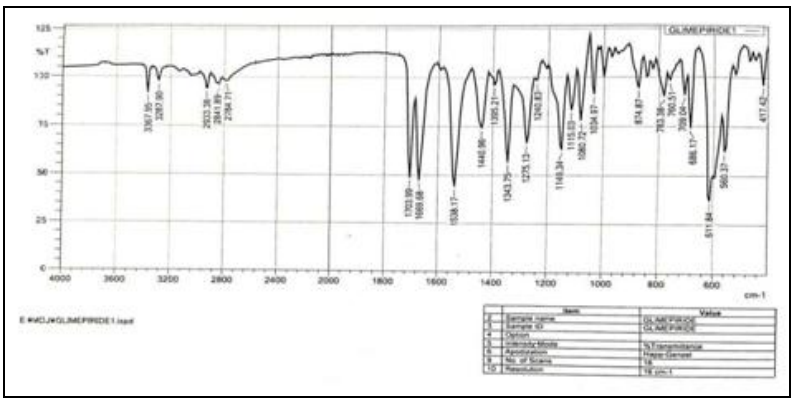

FIG. 6: FTIR SPECTRA OF PURE GLIMEPIRIDE

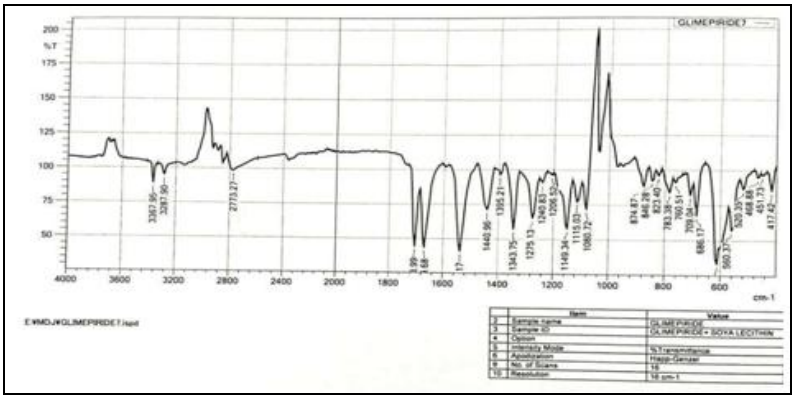

FIG. 7: FTIR SPECTRA OF DRUG+SOYA LECITHIN

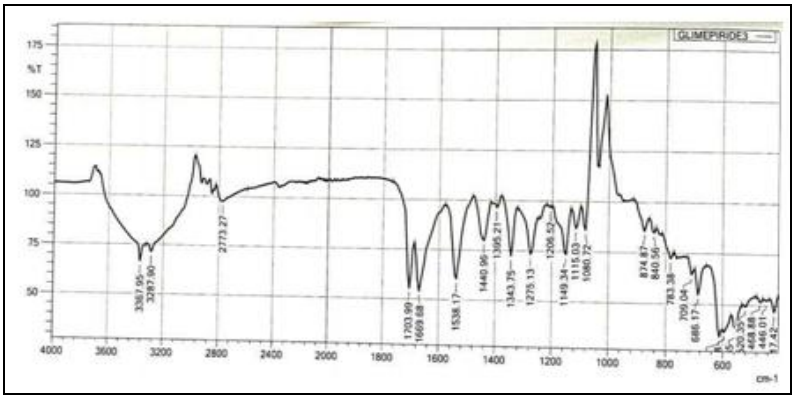

FIG. 8: FTIR SPECTRA OF GLIMEPIRIDE TRANSFEROSOMES

Inference: No chemical interaction and disappearance of peaks between drug and excipients was observed in **Fig. 7, 8.**

Differential Scanning Calorimetric Studies: DSC was used to determine the melting point of Glimepiride. The DSC thermogram of Glimepiride shows a sharp endotherm at 196.98 °C confirming the melting point (**Fig. 9**.

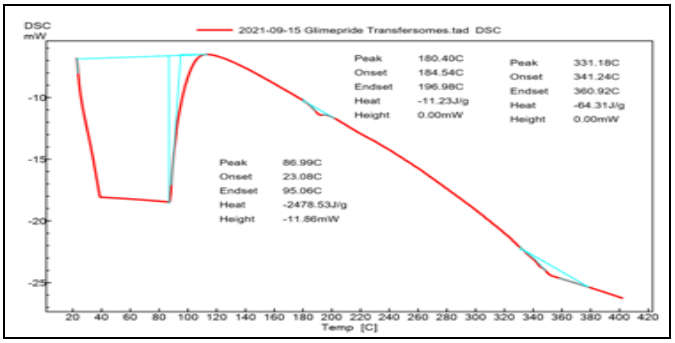

FIG. 9: DSC THERMOGRAM OF GLIMEPIRIDE TRANSFEROSOMES

Characterization of Glimepiride Loaded Transferosomes:

Vesicle Shape and Morphology: The preliminary characterization of Glimepiride-TFS (prior to sonication) was done by using an optical microscope. The optical microscopic images of glimepirde loaded vesicles are shown in **Fig. 10 & 11,** which confirm the formation of vesicles having a spherical shape and different lamellarity **Table 6.**

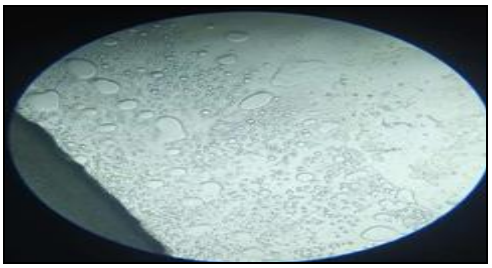

FIG. 10: OPTICAL PHOTOMICROGRAPH OF GLIMEPIRIDE LOADED TRANSFEROSOMES FORMULATION

TABLE 6: MEAN VESICLE SIZE OF GLIMEPIRIDE TRANSFEROSOMES

Formulation code	Size of Vesicle (nm)
F1	353
F2	392
F3	435
F4	448
F5	340
F6	412
F7	362
F8	385
F9	323

The values are expressed as mean, ±SD (n=3)

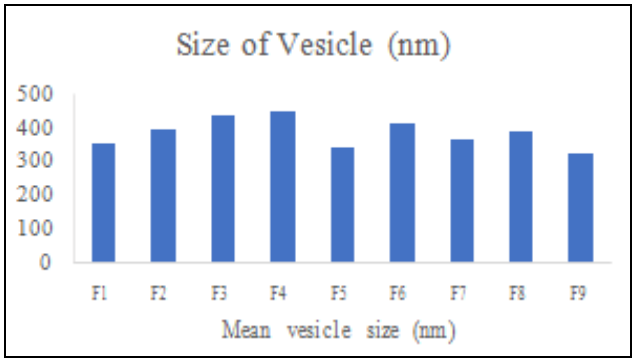

FIG. 11: GRAPHICAL REPRESENTATION OF MEAN VESICLE SIZE

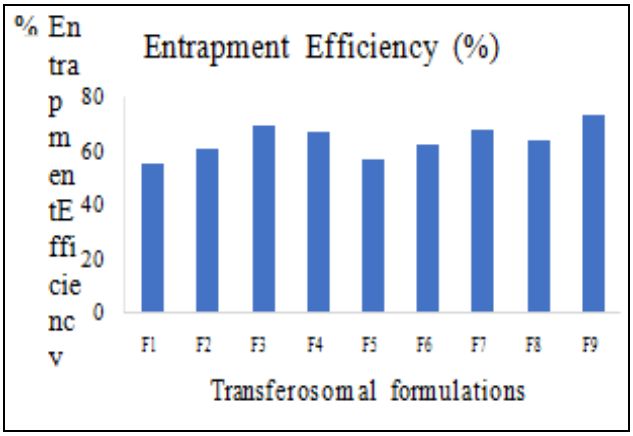

FIG. 12: GRAPHICAL REPRESENTATION OF % ENTRAPMENT EFFICIENCY OPTIMIZATION OF FORMULATION

Entrapment Efficiency (EE):

TABLE 7: % ENTRAPMENT EFFICIENCY OF GLIMEPIRIDE TRANSFEROSOMES

Formulation code	Entrapment Efficiency (%)
F1	55.43
F2	61.25
F3	69.43
F4	67.56
F5	57.15
F6	62.22
F7	68.27
F8	64.35
F9	73.25

The values are expressed as mean, ±SD (n=3)

Inference: The following **Fig. 12 and Table 7** show the Entrapment Efficiency of Vesicles formulation F9 has the highest value.

The central composite design (CCD) was used to find suitable variables. Total 9 experimental runs were executed, and the recorded results are represented in **Table 8.**

TABLE 8: COMPOSITION AND CHARACTERISTICS OF FORMULATIONS

	Factor 1, X_1	Factor 2, X_2	Response 1, Y_1	Response 2, Y_2
Run	A:Soy lecithin	B:Tween 80	Entrapment Efficiency (%)	Size of Vesicle (nm)
1	-1	-1	55.43	353
2	-1	1	61.25	392
3	1	0	69.43	435
4	1	-1	67.56	448
5	-1	0	57.15	340
6	1	1	62.22	412
7	0	1	68.27	362
8	0	0	64.35	385
9	0	-1	73.25	323

TABLE 9: ANOVA FOR QUADRATIC MODEL
RESPONSE 1: ENTRAPMENT EFFICIENCY:

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	276.17	5	55.23	2759.82	< 0.0001	significant
A-Soy lecithin	220.95	1	220.95	11039.73	< 0.0001	
B-Tween 80	51.39	1	51.39	2567.83	< 0.0001	
AB	0.0042	1	0.0042	0.2111	0.6772	
A^2	1.75	1	1.75	87.36	0.0026	
B^2	2.08	1	2.08	103.97	0.0020	
Residual	0.0600	3	0.0200			
Cor Total	276.23	8				

The Model F-value of 2759.82 implies the model is significant. P-values less than 0.0500 in Table 9 indicate model terms are significant. In this case A, B, A^2, B^2 are significant model terms.

Fit Statistics: The Predicted R^2 of 0.9976 is in reasonable agreement with the Adjusted R^2 of 0.9994; *i.e.* the difference is less than 0.2. Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Here ratio of 155.744 indicates an adequate signal.

Std. Dev.	0.1415	R^2	0.9998
Mean	64.32	Adjusted R^2	0.9994
C.V. %	0.2199	Predicted R^2	0.9976
		Adeq Precision	155.7439

TABLE 10: ANOVA FOR QUADRATIC MODEL
RESPONSE 2: SIZE OF VESICLE

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	14629.00	5	2925.80	585.16	0.0001	significant
A-Soy lecithin	12973.50	1	12973.50	2594.70	< 0.0001	
B-Tween 80	1536.00	1	1536.00	307.20	0.0004	
AB	9.00	1	9.00	1.80	0.2722	
A^2	60.50	1	60.50	12.10	0.0401	
B^2	50.00	1	50.00	10.00	0.0508	
Residual	15.00	3	5.00			
Cor Total	14644.00	8				

The Model F-value of 585.16 implies the model is significant. P-values less than 0.0500 Table 10 indicate model terms are significant. In this case A, B, A^2 are significant model terms.

Fit Statistics: The Predicted R^2 of 0.9904 is in reasonable agreement with the Adjusted R^2 of 0.9973; *i.e.*, the difference is less than 0.2. Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Here the ratio of 68.465 indicates an adequate signal. Response analysis through polynomial equations.

Std. Dev.	2.24	R^2	0.9990
Mean	383.33	Adjusted R^2	0.9973
C.V. %	0.5833	Predicted R^2	0.9904
		Adeq Precision	68.4653

Effect of Variables on Entrapment Efficiency: Data was analyzed to fit full second-order quadratic or cubic polynomial equation(s) with added interaction terms to correlate the various studied responses with the examined variables. As depicted by 2D contour plot **Fig. 14A** and 3D response surface plot **Fig. 13A**, the percent entrapment efficiency of drug is positively correlated with X_1, soy lecithin concentration and X_2, tween 80 concentrations. The final mathematical model in terms of coded factors as determined by the Design-Expert software is shown below in Eq. (1) for entrapment efficiency.

Response 1: Entrapment Efficiency, Y_1

$$Y_1 = +64.27 +6.07\ X_1 +2.93\ X_2 -0.0325\ X_1X_2 -0.9350\ X_1^2 +1.02\ X_2^2 \quad \text{…. Eq. (1)}$$

Effect of Variables on size of Vesicle: As depicted in 2D and 3D plots **(Fig. 14B** and **13B)**, it is indicated that at lower levels of soy lecithin, the levels of tween 80 concentration showed a negative influence on particle size. Whereas, increasing the levels of soy lecithin, at a constant tween 80 concentration, an increase in particle size was observed. Thus, the lowest level of soy lecithin and the highest level of tween 80 concentrations resulted in minimum particle size. The final mathematical model in terms of coded factors as determined by the Design Expert software is shown below in Eq. (2) for the size of vesicle.

Response 2: Size of a vesicle, Y_2

$$Y_2 = +383.00 +46.50\ X_1 -16.00\ X_2 -1.50\ X_1X_2 +5.50\ X_1^2 -5.00\ X_2^2 \quad \text{…. Eq. (2)}$$

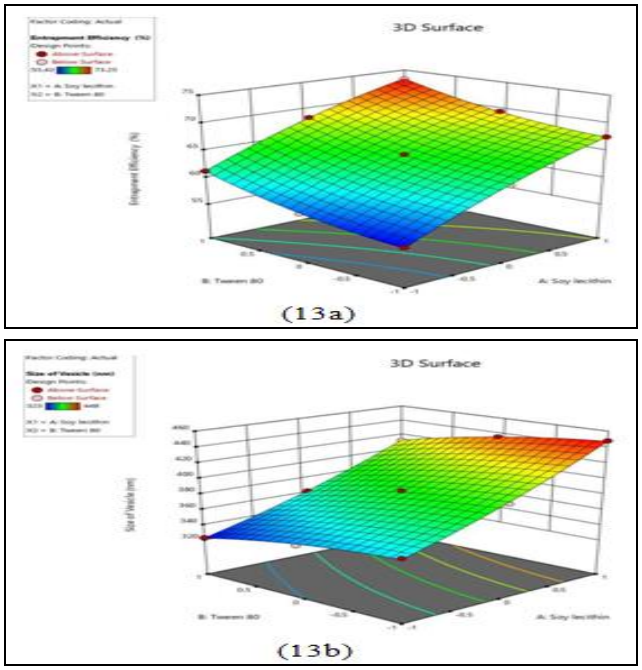

FIG. 13: 3D RESPONSE SURFACE PLOTS FOR EVALUATING THE INFLUENCE OF SOY LECITHIN (X1) AND TWEEN 80 (X2) ON ENTRAPMENT EFFICIENCY (Y1) AND SIZE OF VESICLE (Y2)

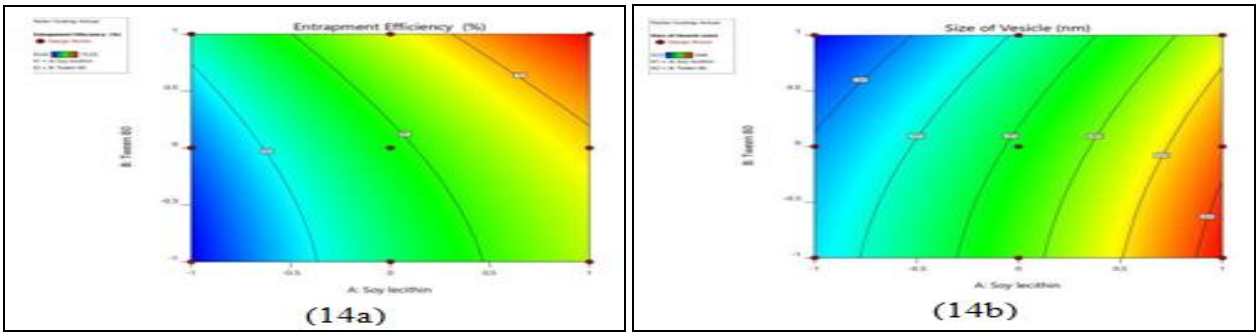

FIG. 14: 2D CONTOUR PLOTS FOR EVALUATING THE INFLUENCE OF SOY LECITHIN (X1) AND TWEEN 80 (X2) ON ENTRAPMENT EFFICIENCY (Y1) AND SIZE OF VESICLE (Y2)

Selection of Optimize Formulation: The optimized Glimepiride loaded transferosomal formulation was chosen from the nine trial formulations prepared according to the factorial design based on the criteria of lowest vesicle size and maximum entrapment efficiency. After a thorough evaluation, it was found that glimepiride-TFS 9 (soya lecithin: 60 mg and Tween 80: 25 mg) fulfilled the requisites of an optimum formulation. The optimized Glimepiride loaded transferosomes GLIM-TFS 9 (OPT-GLIM-TFS) showed 323 nm vesicle size and 73.25 ± 0.32 % entrapment efficiency. The OPT-GLIM- TFS formulation was used for further studies.

Surface Morphology (Vesicle size) of Optimize Formulation: The surface morphology and three-dimensional configurations of the optimized vesicular formulations were further observed by SEM **Fig. 15.** The SEM photographs of transferosomes confirmed their three dimensional (3D) structure and shown the presence of the well-defined spherical or near-spherical shaped vesicles with a smooth surface.

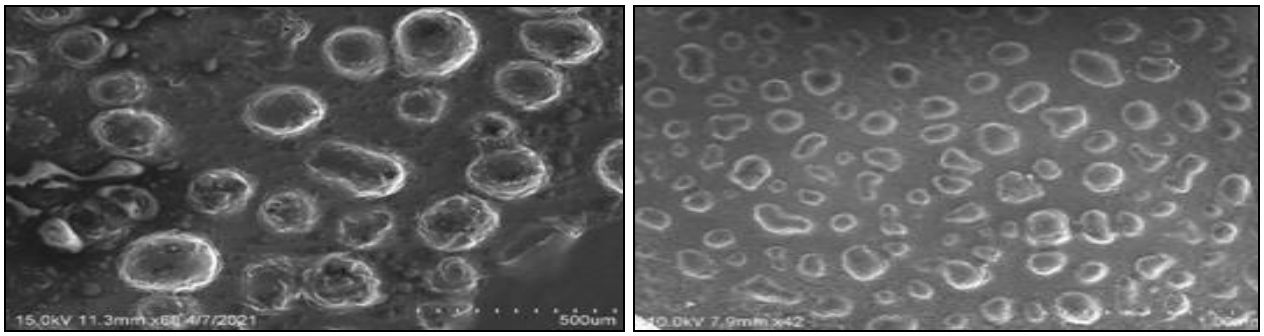

FIG. 15: SEM IMAGES OF GLIMEPIRIDE TRANSFEROSOMES

Zeta Potential of Optimize Formulation: The observed ZP value of optimized formulation was – 23.0 mV for GLIM-TFS **Fig. 16.**

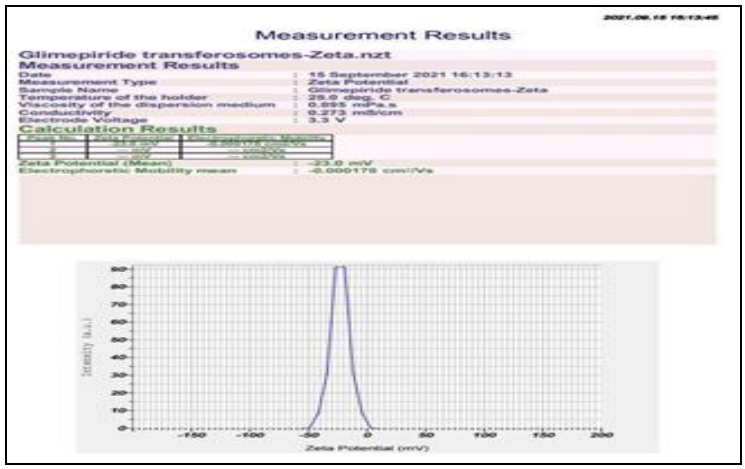

FIG. 16: ZETA POTENTIAL

Determination of *In-vitro* Diffusion of Glimepiride Transferosomes: Diffusion studies of all formulations **in Table 11** were carried out using a dialysis membrane for 10 hours, and samples were analyzed using double beam UV spectrophotometer and were shown in Graphical representation in **Fig. 17.**

TABLE 11: CUMULATIVE % DRUG RELEASE

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Time in hours	**%CDR**	**%CDR**	**%CDR**	**%CDR**	**%CDR**	**%CDR**	**%CDR**	**%CDR**	**%CDR**
0	0	0	0	0	0	0	0	0	0
1	22.84±0.37	17.85±0.43	24.33±0.56	24.00±0.91	28.90±0.35	22.31±0.76	18.13±0.83	18.4±0.54	22.74±0.33
2	34.28±0.43	25.63±0.36	30.10±0.54	26.22±0.81	30.40±0.67	23.12±0.45	35.92±0.76	29.49±0.54	30.21±0.84
3	46.16±0.22	29.66±0.32	33.97±0.53	38.33±0.65	33.20±0.74	35.65±0.43	44±0.83	36.49±0.32	37.38±0.66
4	49.34±0.91	34.69±0.47	38.83±0.45	47.81±0.76	39.21±0.37	40.00±0.68	45.73±0.65	48.53±0.83	49.24±0.61
5	54.64±0.22	40.38±0.12	42.81±0.17	53.34±0.43	40.24±0.66	43.217±0.47	48.06±0.63	50.24±0.76	52.020.32
6	56.25±0.33	45.22±0.78	48.54±0.34	57.31±0.86	43.81±0.57	46.71±0.74	54.23±0.35	52.54±0.56	55.22±0.38
7	59.66±0.37	54.88±0.15	51.22±0.16	62.31±0.47	42.93±0.54	63.43±0.23	57.57±0.65	58.54±0.19	61.37±0.34
8	60.25±0.58	57.65±0.43	64.82±0.86	65.32±0.35	54.42±0.65	66.42±0.76	59.60±0.23	66.24±0.10	69.57±0.18
9	63.54±0.48	62.19±0.48	66.22±0.25	72.40±0.23	60.31±0.44	74.91±0.56	68.11±0.49	70.56±0.54	73.44±0.27
10	74.66±0.45	69.45±0.55	72.15±0.33	75.9±0.27	61.24±0.78	76.64±0.56	70.60±0.51	73.66±0.11	86.05±0.12

The values are expressed as mean, ±SD (n=3).

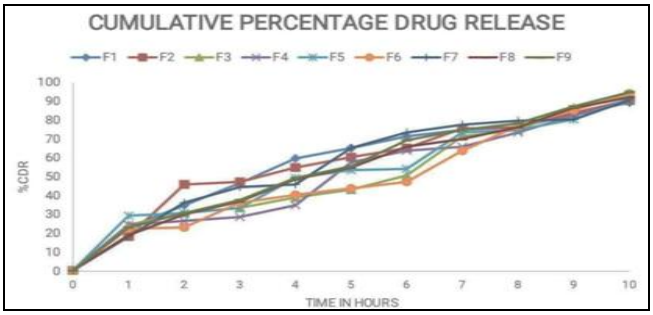

FIG. 17: GRAPHICAL REPRESENTATION OF CUMULATIVE % DRUG RELEASE

Formulation of Glimepiride Transfersomal Gel: Glimepiride Transferosomal gel was prepared using 1.5 % Carbopol 934 as a gelling agent. The concentration of Glimepiride in the prepared Transferosomal gel was 1.5% w/w.

Evaluations of Glimepiride Transferosomal Gel: Glimepiride Transferosomal gel is smooth with a homogenous appearance. The Spreadability value is 12.88±0.50 cm, which indicates that they can be spared easily on the skin surface with little stress. The pH value is 6.4±0.34, which is considered within the normal pH range for topical preparations. The drug content of the glimepiride Transferosomal gel was found to be 86.02±0.21%, representing good content uniformity. The viscosity of GLIM Transferosomal gel is found to be 1656±1.25 cps **Table 12.**

TABLE 12: EVALUATION OF GLIMEPIRIDE TRANSFEROSOMAL GEL

Evaluation	Results
Homogeneity	Good
Spreadability (Gm.cm/sec)	12.88±0.50
pH measurements	6.4±0.34
Drug content (% w/w)	86.02±0.21
Viscosity (cps)	1656±1.25

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

TABLE 13: % CDR, JSS AND KP OF 1.5% OF GLIMEPIRIDE TRANSFEROSOMAL GEL

Formulations	%CDR	Flux (Jss)	Permeability Coeffient (KP)
1.5%Glimepiride transferosomal gel	89.05 + 0.25	4.92	1.021 + 0.54

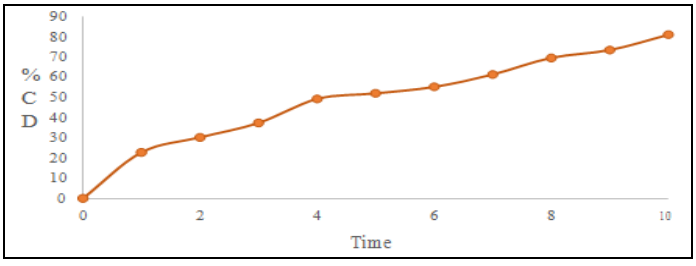

FIG. 18: *IN-VITRO* DRUG RELEASE OF 1.5 % GLIMEPIRIDE TRANSFEROSOMAL GEL

Release Kinetic Profile for Glim Transferosomal Gel: The drug release kinetics studies were estimated to determine the type of release mechanism followed shown in **Table 14 and Fig. 19, 20, 21, 22**. Release kinetic study of GLIM Transferosomal gel of optimized formulation was studied for different kinetic equations (zero order, first order, Higuchi and Peppas equation)

TABLE 14: RELEASE KINETICS FOR 1.5% GLIMEPIRIDE TRANSFEROSOMAL GEL

	Zero	First	Higuchi	Peppas
	% CDR Vs T	Log % Remain Vs T	%CDR Vs √T	Log C Vs Log T
Slope	7.091727273	-0.12191267	25.13428286	1.204854203
Intercept	12.92681818	2.160525354	-2.95309629	0.835777221
Correlation	0.974705137	-0.74576377	0.993980618	0.769989248
R 2	0.939950104	0.983363613	0.987997469	0.592883442

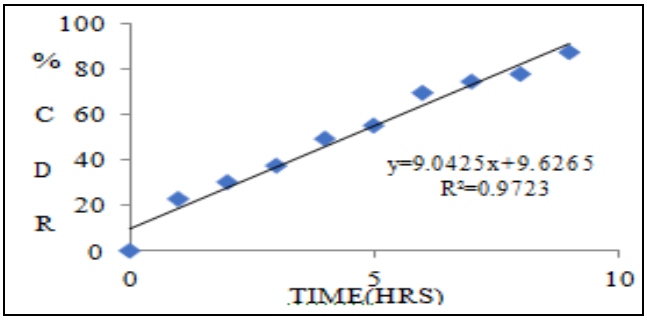

FIG. 19: ZERO ORDER

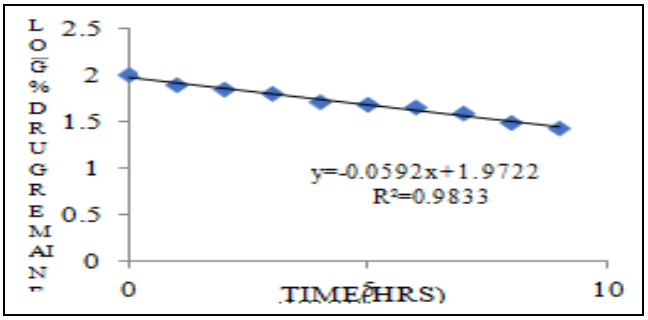

FIG. 20: FIRST ORDER KINETICS

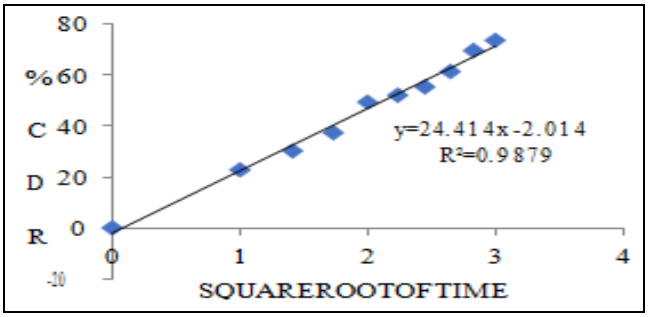

FIG. 21: HIGUCHI MODEL

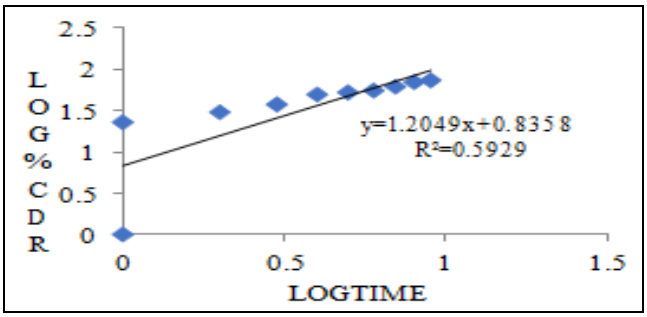

FIG. 22: KORSEMEYERPEPPAS MODEL

DISCUSSION: Glimepiride transferosomal gel formulation shows Higuchi kinetics that indicate the order of drug release of drug i.e, square root of time (Higuchi) model indicates that drug release is by diffusion. R^2 values for the optimized formulation were found to be highest for the zero-order and Higuchi models. This indicated that the drug release from all the formulations followed diffusion controlled release mechanism.

In-vivo Anti-Diabetic Studies:

Serum Glucose Level (mg/dl) Estimation in Rats: The blood glucose concentration were determined for each group by taking blood from the tail vein as per the following schedule; (0^{th}, 1^{st}, 3^{rd}, 7^{th}, 12^{th} days), after the a fore mentioned treatment was given. The blood glucose concentration was determined using the glucometer and the comparisons between the various groups were made.

Pharmacological Studies:

Wound Healing Activity:

Excision Wound Model: Male Albino rats 180-250 gm were taken for studies; the rats were anesthetized before and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using Anaesthetic Ether. A wound of 500 sq. mm on dorsal thoracic region was made. Animals were closely observed **in Table 17 for any infection, and those who** showed signs of infection were separated and excluded from the study and replaced. The animals were observed for wound closure at 0, 4^{th}, 6^{th}, 8^{th}, 12^{th} and 16^{th}day shown in **Fig. 23, 24, 25,** and for a period of epithelialization [25-28].

Measurement of Wound Area: The progressive changes in the wound area were monitored by a 2^{nd}, 4^{th}, 6^{th} ,8^{th} , 12^{th}, and 16^{th}day.

The wound size was also measured using a daily scale, and the wound area was calculated. Wound contraction was calculated as a percentage of the reduction in wound area. (Nayak BS *et. al* 2009).

Percentage of wound contraction = (Initial wound area – Specific day wound area) / Initial wound area × 100

Statistical Analysis: Data analysis was performed using the statistical package for social version 17.0 (SPSS) computer software. Descriptive statistics were adopted to display data in the mean of ± SEM ANOVA was used to compare the mean value obtained between the different groups' **Table 15, Fig. 23.**

TABLE 15: SERUM GLUCOSE LEVEL (MG/DL) IN RATS

Group	Serum glucose level (mg/dl)					
	0 day	1st day	3rd day	7th day	10th day	12th day
Control	88.76±1.21	91.06±1.01	90.46±1.11	92.76±1.21	93.16±1.41	92.11±1.21
STZ	167.6±2.11	264.6±3.11	298.11±4.51	312.1±5.61	324.1±5.21	300.1±5.71
Transferosomes	169.3±3.41	259.76±4.21	289.1±5.21*	283.3±6.21**	171.5±3.21**	151.1±5.21***

Differences were considered significant whenever the P value are reported as mean ± SEM. ***p<0.001, **p<0.01 and *p<0.05.

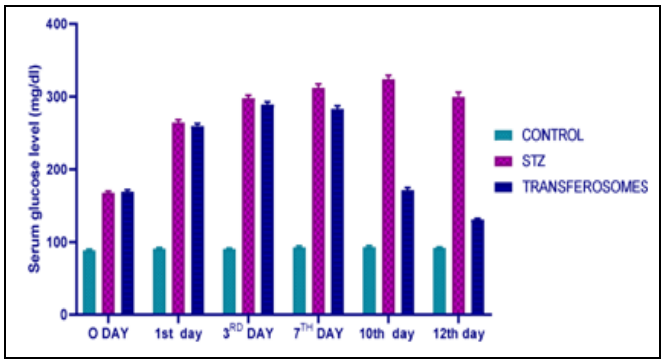

FIG. 23: SERUM GLUCOSE LEVEL (MG/DL) IN RATS

Effect of Glimepiride Transferosomal Gel in Diabetic Wound:

TABLE 16: % WOUND CONTRACTION WITH GLIMEPIRIDE TRANSFEROSOMAL GEL

Treatment	% Wound Contraction with Transferosomal gel							
	1st day	4th day	8th day	12th day	14th day	16th day	18th day	20th day
Control	2.279± 0.839 00.00	2.146± 1.185 8.85	1.928 ± 1.675 19.83	1.581± 1.499 29.67	1.211± 1.700 43.46	1.006± 0.5158 62.81	0.819± 0.11 71.66	0.510± 0.214 82.61
Transfer Osomal Gel	2.259±1.43 00.00	1.927± 1.507 24.69	1.114± 1.054 42.61	0.708± 1.354 88.61	0.397± 0.6750 97.22	-	-	-
Megahel	2.249± 1.198 00.00	1.991± 1.516 23.26	1.547± 1.384 59.65	1.043± 0.8695 81.38	0.720± 0.3782 87.64	0.231± 0.8883 97.69	-	-

Differences were considered significant whenever the P value are reported as mean ± SEM. ***p<0.001, **p<0.01 and *p<0.05.

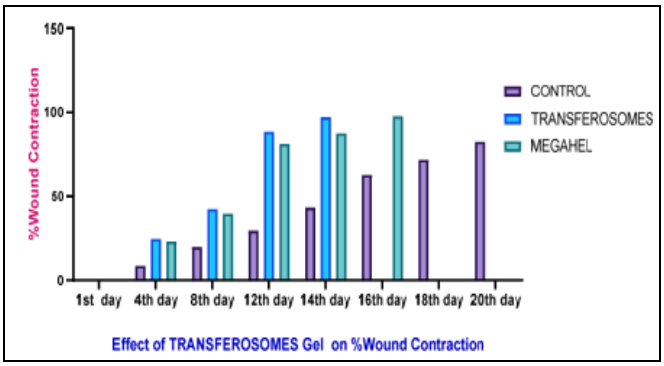

FIG. 24: EFFECT OF GLIMEPIRIDE TRANSFEROSOMAL GEL ON %WOUND CONTRACTION

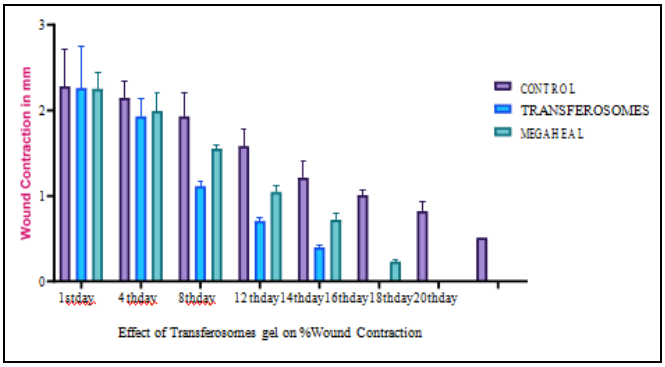

FIG. 25: EFFECT OF GLIMEPIRIDE TRANSFEROSOMAL GEL ON %WOUND CONTRACTION

TABLE 17: WOUND HEALING OF RATS

Days	Groups		
	Group I	Group II	Group III
0 Day			
4th Day			
8th Day			
12th Day			
16th Day			

Stability Studies of 1.5 % Glimepiride Transferosomal Gel:

TABLE 18: STABILITY OF 1.5 % GLIMEPIRIDE TRANSFEROSOMAL GEL AT 4±2°C AND ROOM TEMPERATURE

Duration	4±2°C		Room Temperature	
	DC	%CDR	DC	%CDR
Initial	85.5±1.25	81.6±0.42	86.5±1.41	78.01±0.42
30 days	86.89±1.01	80.45±1.5	84.5±0.54	80.72±0.19
60 days	86.02±0.21	82.32±0.21	81.1±0.84	77.57±0.64
90 days	84.53±1.22	81.44±1.41	79.9±0.16	76.21±1.51

The values are expressed as mean, ±SD (n=3).

Stability studies showed that Glimepiride transferosomal gel is more stable at 4°C compared to other temperatures, as seen in **Table 18.**

CONCLUSION: In the present study, Glimepiride entrapped transferosomes were successfully prepared and optimized using 3^2 factorial designs. The optimized formulations of GLIM-TFS showed a unilamellar vesicular structure with lower vesicle size (323nm) and greater entrapment efficiency (73.25%). The FTIR studies revealed no chemical interaction between drugs and excipients. The *in-vitro* drug release studies of transferosomes were 86.75% of the optimized formulation F9. The optimized formulation of GLIM-TFS was successfully incorporated into carbopol gel and characterized for pH, viscosity, drug content, spreadability.

The % drug release of transferosomal gel was found to be 89.05±0.25, and the kinetic study reveals that drug release follows the Higuchi model, as the R^2 value is highest, which depicts that the release of the drug is by diffusion. It can be concluded that transferosomes can serve as a potential therapeutic strategy for type II diabetes treatment by delivering the Glimepiride into the deepest layers of skin. Further investigation of GLIM-TFS gels in the treatment of type II Diabetes by using suitable animal models was conducted to confirm the efficiency of these formulations. The applicability of these developed vesicular gels for the treatment of diabetes control is being explored. Finally, Stability studies showed that Glimepiride Transferosomal gel is more stable at 4^o C compared to room temperature.

Scope:

- Stability studies can be further extended as per ICH Guidelines.
- IVIVC correlation has to be done.
- Further study in Inflammatory cytokines, serum insulin.
- Study on immune histochemistry of pancreas and liver.

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CONFLICTS OF INTEREST: Nil

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