*Section A-Research paper*

# FORMULATION AND CHARACTERIZATION OF GLIBENCLAMIDE LOADED TRANSFERSOMAL GEL FOR TRANSDERMAL DELIVERY

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## ABSTRACT

Glibenclamide (GBC) is an oral anti hyperglycaemic agent having low bioavailability and it also produces remarkable hypoglycaemia and frequent Gastro Intestinal (GI) side effects. In the present study, Glibenclamide loaded transfersomes were developed for transdermal delivery by modified film hydration method to improve its bioavailability and evaluated for their vesicle size, shape, PDI, Zeta potential, entrapment efficiency, % drug content and *ex vivo* skin permeation study using rat abdominal skin. The optimized formulation F2G consist of Lecithin and Tween 80 at a (lipid: surfactant) ratio of 9:1 showed high entrapment efficiency (98.4%) and vesicle size of 168.9 nm with a Zeta potential of -23.2mV and PDI (0.289). The skin permeation studies of F2G showed that the maximum % drug release (81.2%) and high flux rate of 29.6μg/cm$^2$/h. The flux was showed significantly higher (4.35 times) than the drug solution.

**Key words**: Glibenclamide, Transfersomes, entrapment efficiency, transdermal delivery system, permeation.

## INTRODUCTION

Glibenclamide (GBC) is an oral hypoglycemic drug that stimulates the pancreatic beta cells to secrete insulin and is often used to treat type 2 diabetes, including diabetes during pregnancy It is belongs to sulfonylurea's, it is also known as Glyburide (Dhawan and Singla, 2003). The drug has a plasma half-life 4-6 hrs and needs frequent administration. Moreover, its oral use is associated with severe and sometime fatal hypoglycemic symptoms like nausea, vomiting, heartburn, anorexia and increase in appetite (Goodman and Barry, 1989). Glibenclamide is affected by first-pass metabolism, necessitating high and frequent doses, which results in undesirable side effects. A system of drug input directly into the blood at a constant rate may

lower the high oral dose and minimize side effects **(**Groop et al., 1985).These twin objectives are expected to be fulfilled through Transdermal drug delivery of Glibenclamide..

Transdermal route is more comfortable, convenient and safe method for a patient. This offers several potential advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter-and intra-patient variations, and most importantly, it provides patients convenience. Vesicular systems are one of the most controversial methods for transdermal delivery of drug. Transdermal delivery systems were re-launched the discovery of elastic vesicles such as transfersomes, ethosomes, cubosomes, phytosomes, liposomes, Proniosomes etc.

Transfersomal concept was introduced in 1991 by Cevc (1996). The vesicular approaches such as penetration enhancers (Karande and Mitragotri, 2009), Iontophoresis (Abla et al., 2006) and lipid vesicle carriers (Shah at al., 2007; Dragicevic curic et al., 2008) have been used to alter skin to allow the transport of therapeutic agents across it. The elastic deformable vesicles serve as penetration enhancers that can structurally modify by lipids and deeply penetrate through the skin with minimal risk of vesicular wall rupture compared to conventional niosomes and liposomes (Muzzalupo et al., 2011; Kakkar and Kaur, 2013). Novel transfersomes composed with various types of edge activators (Eas). Being surfactant, they can lower the surface tension of vesicular lipid bilayer and consequently increase their deformability (Duangjit et al., 2013). So that they enter by systemic uptake via dermal blood vessels and consequently bypass the gastrointestinal side effects associated with oral rote (EI Zaafarany et al., 2010)
Transdermal gel formulation overcome the first pass metabolism and improves the bioavailability of the drug. The purpose of the investigation was to formulate a stable Transfersomal gel of Glibenclamide for transdermal delivery and evaluate its drug permeation with comparison to transfersome suspension and plain drug suspension.

## MATERIALS AND METHODS

Glibenclamide was obtained as a gift sample from Sri Krishna Pharmaceuticals Ltd., Hyderabad, India. Tween 80 and Span 80 were obtained as a gift samples from Sigma-Aldrich Chemicals Private Ltd. Hyderabad, India. Egg lecithin was procured from TCI Chemicals (India) Pvt. Ltd., Hyderabad, India. Ethanol and Dichloromethane were purchased from S.D. Fine Chemicals, Mumbai, India and Methanol was procured from High Pure Fine Chemicals, Chennai, India.

### DRUG CHARACTERIZATION BY DSC:

The purity of drug was determined by Differential Scanning Calorimetry (DSC 4000, PerkinElmer). For this, about 5-10 mg of drug was taken in a pierced aluminum pan and crimped it well; the melting point was noted at which the material was melted at a temperature range of 50-200°C. The heating rate was kept constant at 10°C/min for entire process. The nitrogen gas

served as purged gas and empty aluminum pan was used as reference cell for this study (Jessy Shaji et al., 2013). The DSC thermogram of pure drug was shown in Fig.1.

**SOLUBILITY STUDIES**

An excess amount of drug was taken in a screw capped vials containing solvents, surfactants and buffers and the contents were shaken for 48 hr by using gyratory shaker at room temperature. The supernatant was collected after centrifugation and filtered it using 0.22 µ membrane filter. The Samples were diluted suitably and the absorbance was measured using UV-Vis spectrophotometer at 229nm (Nagadeep J et al., 2019). The solubility of Glibenclamide in various surfactants, buffer and solvents was given in Table 1.

**PREPARATION METHOD OF GLIBENCLAMIDE LOADED TRANSFERSOMAL GEL**

***Preparation of Transfersomes by thin film hydration method***

Transfersomes were prepared by thin film hydration method using lipid (egg lecithin), surfactant (Tween 80) and ethanol: DCM (2:1) mixture as a solvent. The required amount of egg lecithin and Tween 80 was taken in a round bottom flask and dissolved in mixture of ethanol: DCM solvent. Then, the solution was evaporated in rotary flash evaporator at 40ºC and 60 rpm to form thin film. The film was hydrated with pH 7.4 phosphate buffer, containing drug. The resulting vesicles allowed to swelling at room temperature for 2 hrs to form LMLVs (large multi-lamellar vesicles). The formed suspension was subsequently sonicated for 5 min using probe sonicator to form small uni-lamellar vesicles (SULVs). Finally, the formulation was stored under refrigeration (Jaishetty et al., 2016). The composition of transfersomes was given in Table 1.

***Preparation of Transfersomal gel***

Transfersomal gel was prepared by incorporation of Transfersomal suspension into a structured vehicle such as Carbopol polymer. Carbopol gel was prepared by adding Carbopol 934 (1.5%w/v) to water with continuous stirring by using magnetic stirrer and allowed it to hydrate for 12 hrs and adjusted the pH 6.8 with triethanolamine using pH meter (ELICO LI120). Finally, the optimized formulation was added to the gel drop wise and allowed to swollen to obtain a homogenous gel (Jaishetty et al., 2019).

**Table 1. Composition of Glibenclamide loaded transfersomes**

| **Ingredients (mg)** | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** |
|---|---|---|---|---|---|---|
| **Glibenclamide** | 5 | 5 | 5 | 5 | 5 | 5 |
| **Tween 80** | 5 | 10 | 20 | 30 | 40 | 50 |
| **Egg lecithin** | 95 | 90 | 80 | 70 | 60 | 50 |
| **Ethanol: DCM** | 3 | 3 | 3 | 3 | 3 | 3 |
| **Phosphate buffer (pH 6.8) (ml)** | 5 | 5 | 5 | 5 | 5 | 5 |

**DCM: Dichloromethane**

## EVALUATION OF TRANSFERSOMES

### Determination of Mean Globule Size, Zeta potential and PDI

The globule size, zeta potential and PDI of transfersomes was measured by using Malvern Zetasizer (Nano ZS 90). The samples were prepared by diluting 50 times with double distilled water and taken in a cuvette; the globule size was measured at a light scattering angle of 90º. Three readings were noted for every sample and the mean globule size was computed. For zeta potential and PDI, the sample was taken in a dip cell, inserted into Zetasizer and the average of three readings was computed (Syeda Shabana Sultana et al., 2015).

### Surface Morphology by SEM

The surface morphology of transfersomes was observed by using scanning electron microscope (SEM S-3700N, Hitachi Ltd, Japan). About 0.2-0.5 g of transfersomes was taken in a glass tube and it was mounted on an aluminum stub using double sided adhesive carbon tape. Then, the vesicles were super coated with gold palladium using a vacuum evaporator and examined using a SEM equipped with a digital camera at an accelerating voltage of 15 KV (Kavitapu et al., 2023).

### Determination of % drug content

The transfersomal suspension equivalent to 1g was taken and diluted with 10 mL of methanol in a volumetric flask and the amount of drug was estimated by UV-Vis spectrophotometer at 229 nm against methanol as a blank. The absorbance readings were noted triplicate and % drug content was computed using suitable formula (Alwera et al., 2022).

### Determination of Entrapment Efficiency

The transfersomal dispersion equivalent to 5mg of drug was taken in a dialysis sac and soaked it in 7.4 pH phosphate buffer at room temperature for 8 hrs. The suspension was centrifuged at a temperature of 4 °C using cooling centrifuge after soaking. The free drug concentration in the resulting supernatant and the resulting washing solution was assayed spectrophotometrically at 229 nm after filtration and suitable dilution. The percentage of drug entrapped was calculated by using the following formula: (Nagadeep et al., 2021, Nadia M. Morsi et al., 2016)

%Entrapment efficiency = [total drug added-unentrapped drug (i.e. free drug)/ total drug added] × 100

### Measurement of pH and Viscosity

The pH was determined using digital pH meter (Remi, India). 1g of gel was taken in a beaker and the pH was noted by dipping the glass electrode completely into gel system to cover the electrode and adjusted the pH to 5.5- 6.5. The viscosity of gel was measured using Brook field viscometer. The viscosity was noted by using spindle no.64 at 20 rpm and the corresponding dial reading was noted (Yasmeen et al., 2022)

### *In vitro* drug release studies

An *in vitro* drug release study was performed using Franz diffusion cell. Dialysis membrane (Hi Media, Molecular weight 5000 Daltons) was placed between receptor and donor compartments.

Transfersomal gel of equivalent to 5 mg of drug was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4. The diffusion cells were maintained at 37±0.5°C with stirring at 50 rpm throughout the experiment. At different time interval, 2 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed for drug content by UV Visible spectrophotometer (Yameen et al., 2022)

***Ex vivo* Skin Permeation Studies**

The rat abdominal skin and Franz diffusion cell was used for the *ex vivo* permeation study. The male Wistar rats weighing 250 ± 20 g were used in this study. The full thickened skin free of bites and scratches was excised after removing hair with an electronic shaver. The subcutaneous fat was carefully removed without damaging the epidermis by placing in hot water at 60°C for 30 seconds. The skin was washed with phosphate buffer (pH 7.4) and inspected for its integrity under microscope. The skin was placed on donor compartment of Franz diffusion cell as the stratum corneum facing upward and dermal side facing downward (receptor compartment). The sample (1 ml) of transfersomal formulation equivalent to 5 mg of drug was accurately weighed and placed on the skin mounted on diffusion cell. The receptor compartment was filled with 20 ml phosphate buffer (pH 7.4) and the entire set was placed on magnetic stirrer and the permeation studies were performed at room temperature with stirring rate of 100 rpm. The aliquots (2ml) were withdrawn from the receptor compartment at various time intervals up to 8 hrs and replaced with an equal volume of fresh buffer. Then, the samples were assayed for the amount of drug permeated by using UV-Visible spectrophotometer at 229 nm. The significant *p* values were calculated by Graph Pad prism software version 8. The following formula was used to calculate cumulative amount of drug permeated through abdominal skin and the experiments were carried in triplicate. (Muhammad Imran Khan et al.,2022, Anjali Devi Nippani et al.,2018)

$$Q = [C_n V + \sum_{i=1}^{n-1} C_i S]$$

.

*Where*, Q= Cumulative amount of drug permeated (µg); $C_n$= Concentration of drug (µg/ml) in $n^{th}$ sample interval; V= Volume of Franz diffusion cell (20 mL), S= Sampling volume (2mL)

n-1

$\Sigma C_i S$ = Sum of drug concentration of sample (1 to n-1)

i =1 multiplied with sampling volume (S)

**Drug-Excipient compatibility studies**

The drug excipient compatibility studies were carried out using FTIR (Bruker FT-IR Tensor 27) by KBr disc method. The spectrum was obtained at a resolution of 4 $cm^{-1}$ in the frequency range of 4000–400 $cm^{-1}$. The spectrums obtained for pure drug and optimized formulation (F2) were shown in Fig.2 & Fig.3 respectively (Neha Thakur et al., 2018)

**Stability studies**

The optimized formulation of transfersomal gel was taken in an amber colored glass bottles and subjected to accelerated stability testing at both refrigeration temperature and room temperature. The analysis of the samples was characterized for vesicle size and drug content after a period of 7, 14 and 28 days (Yameen et al., 2022)

## RESULTS AND DISCUSSION

**Drug characterization by DSC**

The DSC thermogram of a Glibenclamide shown in Fig.1 and it showed endothermic peak at 168.63ºC which corresponds to theoretical melting point of Glibenclamide (160.7 ºC). From the thermogram, it indicated that the drug is pure and in crystalline form.

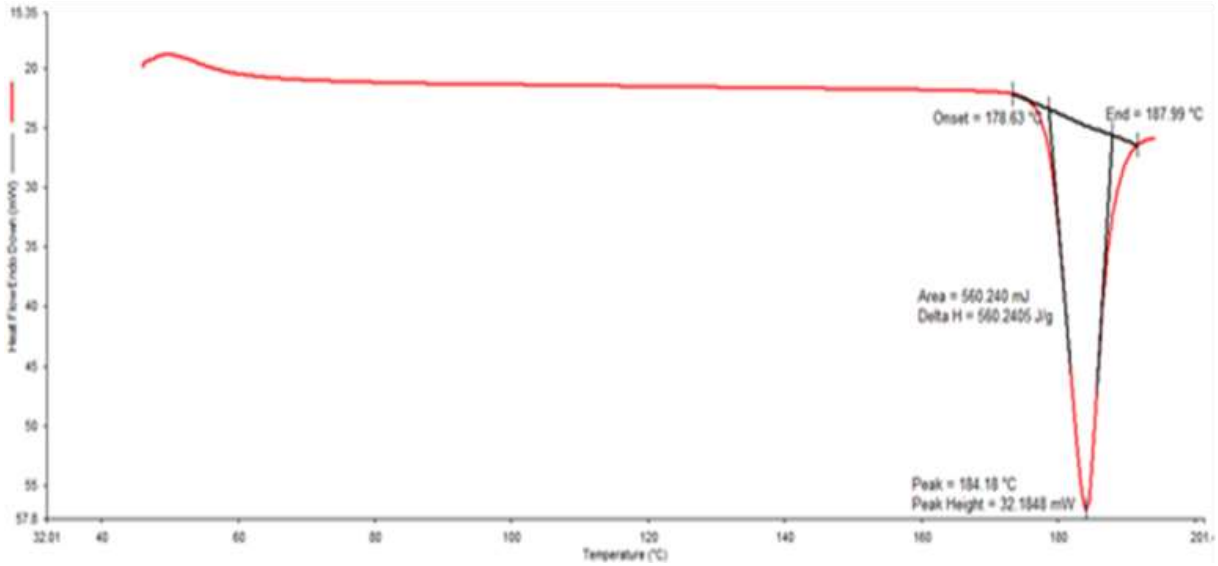


**Fig.1: DSC thermogram of Glibenclamide**

**Solubility Studies**

Solubility of Glibenclamide was studied in various surfactants, buffers and solvents and the results were given in Table 2. The solubility of Glibenclamide was varied from 0.9±0.02 to 59±0.93 mg/ml in various solvents. The highest solubility was found to be 59±0.93 mg/ml in dichloromethane followed by Tween 80 (49.2±0.87 mg/ml) whereas least was found to be 0.9±0.02 mg/ml in water. Hence, the dichloromethane and Tween 80 were used in the development of transfersomes.

**Table.2: Solubility of Glibenclamide in various solvents**

| S.No. | Vehicle | Solubility (mg/ml) |
|---|---|---|
| 1 | Span 80 | 19.4±0.71 |
| 2 | Span 20 | 14.21±0.23 |
| 3 | Tween 60 | 21.4±0.76 |
| 4 | Tween 80 | 49.2±0.87 |
| 5 | Water | 0.9±0.02 |
| 6 | DCM | 59±0.93 |
| 7 | PEG 400 | 28.12±0.82 |
| 8 | Ethanol | 38±0.85 |

**DCM: Dichloromethane; PEG: Polyethylene glycol**

**The characterization of transfersomes**

The mean globule size of all transfersomes was found to be in the range of 169.5±3.4 to 757.7±5.2 nm. The results indicated that the globule size was increased with increased surfactant concentration. This may be due to the less surface activity of surfactant at high lipid concentration. The zeta potential values were varied in the range of -23.1±0.2 to -42.8±1.3. The formulation F2 showed the lowest mean globule size of 169.5±3.4 nm, Zeta Potential of -23.1 mV and PDI of 0.281. The % drug content was found to be within the range of 85.9±0.74 to 96.2±0.92. The size, zeta potential &PDI and % drug content results were shown in Table 3. Based on the globule size, F1, F2& F3 formulations were selected for further preparation of gel by adding Carbopol 934 (1%w/v, 1.5%w/v).

**% Entrapment Efficiency**

Entrapment efficiency is the percent of the total drug incorporated into the transfersomes. The entrapment efficiency was found between 90.3±0.54 to 98.9±0.57% and shown in Table 3. The F2 and F3 formulations showed higher entrapment efficiency than all other formulations. The entrapment efficiency in transfersomes largely depends on the concentration of surfactant in the bilayer. Initially, with increasing surfactant concentration, there was an increase in entrapment efficiency (up to 20%). Then after a threshold level (i.e. above 20% of surfactant), a further increase in surfactant concentration led to a decrease in entrapment efficiency. This may be due to the fact that the surfactant molecule gets associated with the phospholipid bilayer, resulting in better partitioning of drug. So above a 20% concentration of the surfactant, molecules may start forming micelles in a bilayer resulting in pore formation in vesicle membranes and complete

conversion of vesicle membranes into mixed micelles. These mixed micelles were reported to have a lower drug carrying capacity and poor skin permeation due to their structural features.

**Table 3: The characterization of transfersomes**

| Formulation Code | Lipid: surfactant | Size (nm) | PDI | Zeta potential (mv) | %Drug content | % Entrapment efficiency |
|---|---|---|---|---|---|---|
| **F1** | 95:05 | 226.8±2.6 | 0.316±0.05 | -34.7±1.3 | 85.9±0.74 | 97.4±0.63 |
| **F2** | 90:10 | 169.5±3.4 | 0.281±011 | -23.1±0.2 | 96.2±0.92 | 98.9±0.95 |
| **F3** | 80:20 | 249.8±2.8 | 0.398±0.12 | -27.4±0.8 | 92.1±0.86 | 98.4±0.57 |
| **F4** | 70:30 | 376.2±3.6 | 0.562±0.06 | -42.8±1.3 | 89.4±0.82 | 95.9±0.61 |
| **F5** | 60:40 | 552.7±4.2 | 0.621±0.15 | -28.9±0.6 | 93.0±0.90 | 91.7±0.50 |
| **F6** | 50:50 | 757.7±5.2 | 0.435±0.24 | -30.5±1.7 | 83.9±0.79 | 90.3±0.54 |

**Table 4: pH and Viscosity of Transfersomal gel**

| Formulation Code | pH | Viscosity (cP) |
|---|---|---|
| **F1G** | 6.4± 0.5 | 43782 |
| **F2G** | 6.5± 0.1 | 48682 |
| **F3G** | 6.2± 0.3 | 42976 |

The pH of formulations plays an important role in transdermal delivery, the pH of the Transfersomal gel formulations was in the range of 6.2 ± 0.3 to 6.4± 0.5 which lies in the normal pH range of the skin and therefore it will not induce any skin irritation and the viscosity was measured by using Brookfield viscometer and The viscosity of formulations was found to be 42976 to 48682 cP. All formulations were showed good gel properties. All pH and Viscosity results were shown in Table 4.

**Surface morphology study**

Fig.2 represents the photomicrographs of the optimized formulation F2G by SEM. It shows the outline and core of the well-identified spherical shaped vesicles, displaying the retention of sealed vesicular structures, which are nearly homogenous in shape.

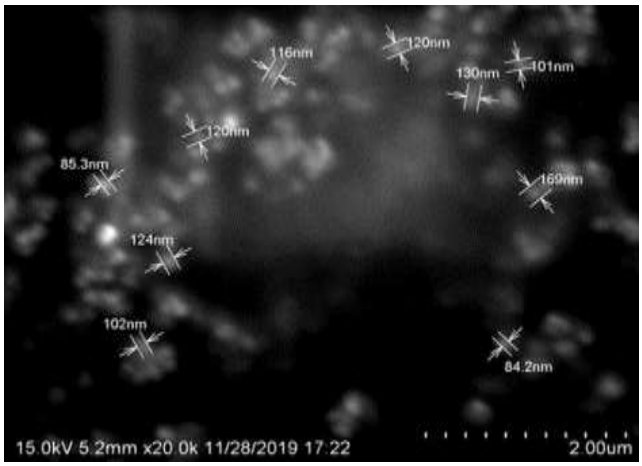


**Fig 2: SEM image of optimized formulation F2G**

***In vitro* drug release study**

*In-vitro* release study of the transfersomal formulations including gels and drug suspension performed using Franz diffusion cell with dialysis membrane in phosphate buffer pH 7.4 for a period of 8 hours. The data obtained from diffusion studies are summarized in Table 5. The figure 3 showed the comparative cumulative % drug release. The results showed that the transfersomal gels released drug in controlled release manner in 8 hour when compared to drug suspension and formulations without gelation. The gel formulation F2G showed the highest % drug release of 81.3% compared to other formulations.

**Table 5: *In vitro* drug release data**

| **Time (hr.)** | **% Cumulative drug release** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **F1G** | **F2G** | **F3G** | **F1** | **F2** | **F3** | **Drug Suspension** |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 8.9±1.6 | 11.2±0.89 | 6.1±2.1 | 4.5±0.69 | 4.9±1.38 | 3.1±0.89 | 0.58±0.56 |
| 2 | 16.7±0.9 | 23.8±1.33 | 10.7±3.6 | 9.8±0.98 | 9.3±0.82 | 7.5±3.17 | 0.92±0.42 |
| 3 | 23.6±2.1 | 34.3±2.75 | 19.2±1.28 | 12.3±2.16 | 13.6±0.67 | 11.2±1.22 | 1.6±0.61 |
| 4 | 30.5±1.9 | 43.1±0.79 | 28.5±0.97 | 15.9±1.60 | 19.4±1.17 | 14.6±0.76 | 2.5±0.32 |
| 5 | 39.7±2.9 | 52.6±1.89 | 33.4±0.89 | 19.4±2.10 | 25.7±2.72 | 17±1.86 | 3.8±0.97 |
| 6 | 47.1±3.5 | 68.7±2.36 | 40.8±2.11 | 25.4±3.16 | 33.8±2.89 | 21.3±2.08 | 4.9±1.1 |
| 7 | 56.9±2.3 | 72.6±3.15 | 48.2±3.97 | 29.3±2.08 | 42.9±3.22 | 26.7±3.21 | 6.7±1.6 |
| 8 | 63.4±4.1 | 81.3±3.88 | 52.8±4.31 | 32.5±3.87 | 56.8±3.51 | 30.5±4.42 | 8.4±1.03 |

Each value represents mean±SD

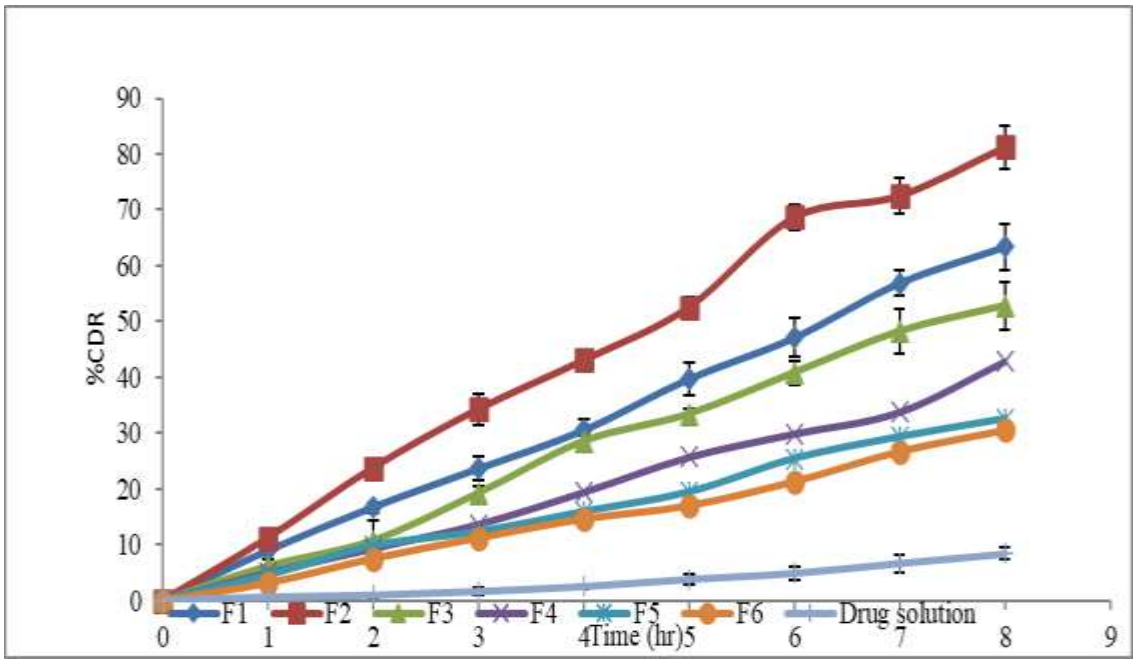


**Fig 3: *In vitro* drug release profiles of transfersomes and drug solution**

***Ex vivo* permeation studies**

The *ex vivo* skin permeation studies were performed by using Franz diffusion cells in 7.4 pH phosphate buffer. The amount of drug permeated and flux values of the Transfersomal formulations as well as drug suspension were shown in Table 6 & 7 respectively. The amount of drug permeated from transfersomes shown Fig 4. The All the transfersomal gel formulations showed significantly high ($p<0.0001$) permeation (flux) compared to drug suspension. The steady state flux of F2G found to be high flux of 29.6±1.08 μg/cm$^2$/hr compared to F2 (17.4±1.02 μg/cm$^2$/hr) and drug solution (6.9 ±1.01 μg/cm$^2$/hr). The formulations F2, F2G showed 2.55, 4.35 times more flux values than drug suspension respectively. This is due to the fact that enhanced partitioning of vesicles into the stratum corneum under the influence of the trans-epidermal osmotic gradient which exists for longer period in gel formulation compared to Transfersomal formulation. These findings were in accordance with Kirjavainen et al. 1999 and Hema Chaudhary et al. 2010. The comparative flux of transfersomes and drug solution was shown in Fig 5.

**Table 6: *Ex vivo* permeation studies**

| Time | Amount of drug permeated (μg) | | | | | | |
|---|---|---|---|---|---|---|---|
| ( hr) | F1G | F2G | F3G | F1 | F2 | F3 | DS |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 457.29 ±1.6 | 841.12 ±0.89 | 421.31 ±2.1 | 465.12 ±1.58 | 495.21 ±0.78 | 412.32 ±1.58 | 281.32 ±0.56 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2 | 532.12 ±0.9 | 1029 ±1.33 | 495.12 ±3.6 | 498.42 ±1.43 | 512.2 ±1.73 | 465.2 ±2.11 | 454.32 ±0.42 |
| 3 | 642.3 ±2.1 | 1144.12± 2.75 | 598.32 ±0.97 | 598.16 ±1.02 | 612.12 ±2.5 | 565.2 ±1.47 | 512.34 ±0.61 |
| 4 | 989.12 ±1.9 | 1603.21 ±0.79 | 892.12 ±0.89 | 792.3 ±2.47 | 1021.31 ±2.45 | 712.35 ±1.92 | 612.89 ±0.32 |
| 5 | 1521.12 ±2.9 | 1702.12 ±1.89 | 1124.12 ±2.11 | 1102.19 ±2.63 | 1523.12 ±1.96 | 1065.34 ±0.99 | 842.31 ±0.97 |
| 6 | 2154.12 ±3.5 | 2312.12 ±2.63 | 1834.12 ±3.97 | 1532.12 ±1.59 | 1989.54 ±2.37 | 1359.12 ±2.38 | 952.32 ±1.1 |
| 7 | 2566.12 ±2.3 | 3612.12 ±3.15 | 2121.12 ±2.11 | 2013.75 ±1.37 | 2298.35 ±3.16 | 2019.12 ±3.89 | 1235.2 ±1.6 |
| 8 | 5342.12 ±4.1 | 4092.12 ±3.88 | 3012.34 ±4.31 | 2965.89 ±3.31 | 3212.12 ±3.64 | 2645.21 ±3.25 | 1956.32 ±1.03 |

Each value represents the mean ±SD (n=3)

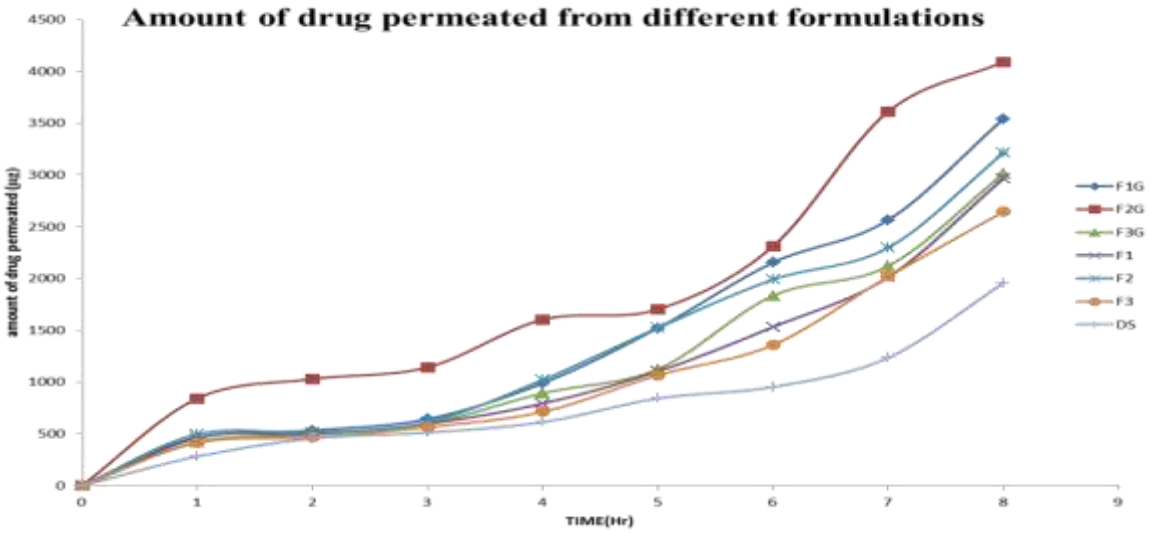


**Fig 4: *Ex vivo* permeation profiles of transfersomal formulations and drug solution**

**Table 7.Flux values of various formulations and drug suspension**

| Formulation Code | Flux (µg/cm$^2$/hr) | Kp*10$^{-3}$ | Enhancement Ratio |
|---|---|---|---|
| **F1G** | 25.7 | 5.14 | 3.77 |
| **F2G** | 29.6 | 5.92 | 4.35 |

| | | | |
|---|---|---|---|
| **F3G** | 19.8 | 3.96 | 2.91 |
| **F1** | 15.3 | 3.06 | 2.25 |
| **F2** | 17.4 | 3.48 | 2.55 |
| **F3** | 12.1 | 2.42 | 1.77 |
| **Drug Suspension** | 6.8 | 1.36 | 1 |

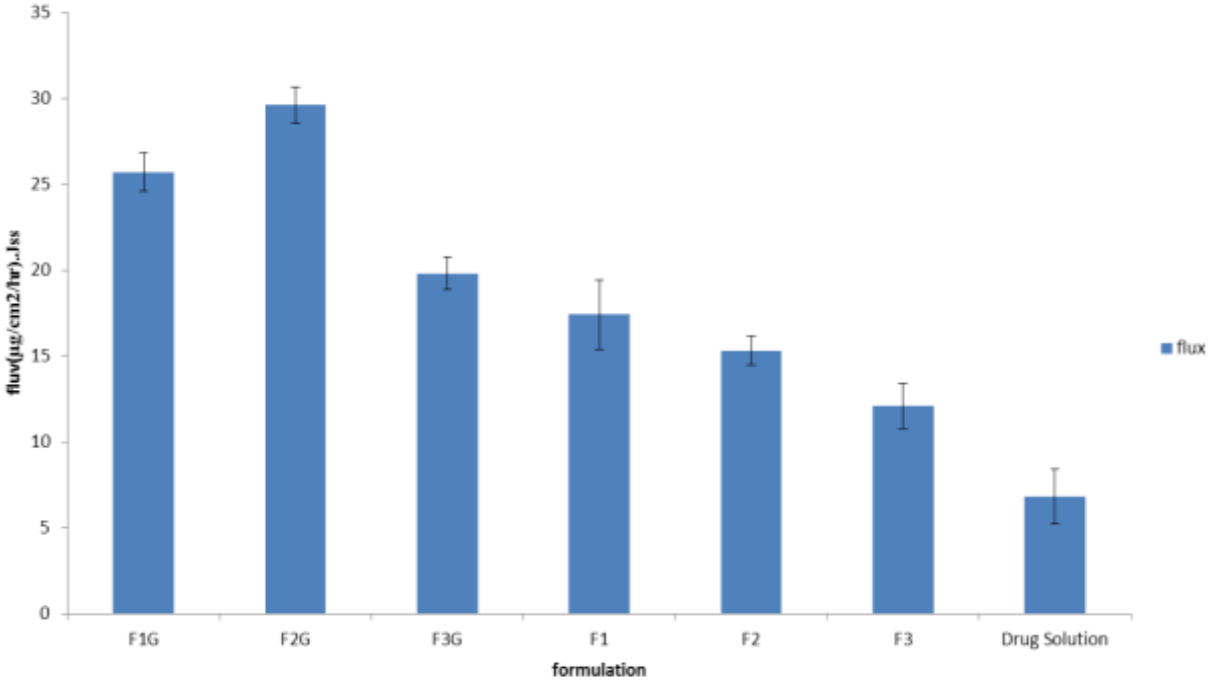


**Fig 5: Steady State Flux of Transfersomal formulations and drug solution**

**Drug Characterization by FTIR**

FTIR studies were carried out to confirm the compatibility between the lipid, drug, and selected excipients. The FT-IR spectra of Glibenclamide and Transfersomal gel F2G formulation were shown in Fig 6a & Fig 6b respectively. From the spectra it was observed that there was no major shifting, as well as, no loss of functional peaks between the spectra of the drug and transfersomes gel. This indicated no interaction between the drug and other excipients

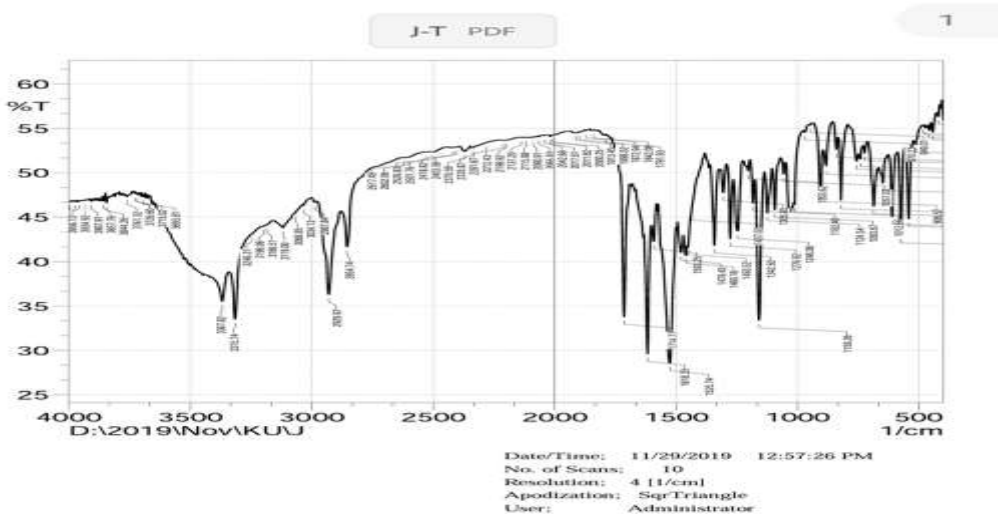


**Fig 6a. FTIR spectra of Glibenclamide**

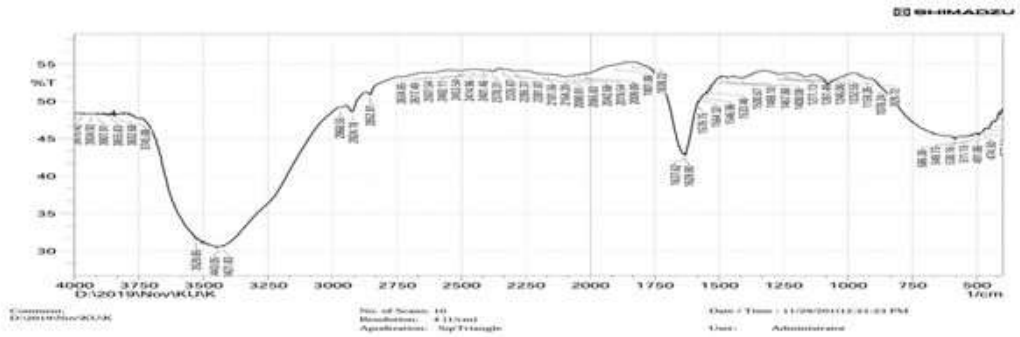


**Fig 6b. FTIR spectra of optimized formulation (F2G)**

**Stability studies**

Stability studies for optimized formulation F2G were carried out at 4.0 ± 0.5$^{o}$C and 25 ± 0.5$^{o}$C for a period of four weeks. There was no significant variation found in physical appearance, average particle size and % drug content of the transfersomes gel. The results were shown in Table 8.

**Table 8: stability data of optimized F2G formulation**

| Days stored | Storage temperature | | | |
|---|---|---|---|---|
| | At 4.0 ± 0.5$^{o}$C | | At 25 ± 0.5$^{o}$C | |
| | Size (nm) | %drug content | Size (nm) | %drug content |
| **Initial** | **169.5±3.4** | **96.2±0.92** | **169.5±3.4** | **96.2±0.92** |
| **7** | 168.7±2.1 | 96.0±0.87 | 170.2±2.8 | 95.9±0.89 |
| **14** | 168.5±1.4 | 95.8±0.90 | 168.3±2.1 | 95.5±0.85 |
| **28** | 167.2±1.5 | 95.1±0.82 | 167.5±1.9 | 95.2±0.83 |

## CONCLUSION

In present study an attempt was made to entrap Glibenclamide into transfersomes by Thin Film Hydration method. Further transfersome suspension is converted into Transfersomal gel using Carbopol 934 as gelling agent.

Among all Transfersomal formulations, F2 showed good characterization of transfersomes with high % entrapment efficiency (98.9±0.95) and smaller particle size (169.5±3.4 nm). The SEM of optimized F2 Transfersomes appeared as spherical, well identified, unilamellar nanovesicles. The optimized formulation of Transfersomes (F2) was further formulated to gel using Carbopol at 1.5% w/w. The F2G gel formulation further increased the permeation of drug (4092.12±3.88 µg) and % drug release (81.3±3.88) when compared to F2 formulation and drug solution. F2G gel showed 2.55 and 4.35 times more flux (29.6±1.08 µg/cm$^2$/hr) when compared to F2 (17.4±1.02 µg/cm$^2$/hr) and drug solution (6.9 ±1.01 µg/cm$^2$/hr) respectively. The pH, viscosity was found to be most suitable for transdermal application. The stability studies showed that Transfersomal gel is more stable at both refrigeration and room temperature for 28 days.

By the present investigation we can concluded that F2G transfersomal gel formulation composed of 5% w/w of Glibenclamide, Egg lecithin: Tween 80 at 9:1 ratio and 1.5%w/w of Carbopol showed good physicochemical properties with significant flux enhancement and the transfersomal gel has the ability to overcome the barrier properties of the skin and increase the drug release. Hence, the transfersomal gel of Glibenclamide for transdermal delivery is promising alternative to oral preparations by avoiding first pass metabolism and thus improving the bioavailability.

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**Conflict of Interest**

The authors have declared no conflict of interest.

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