Research Article

Glibenclamide loaded ethosomal gel for transdermal delivery: Formulation, optimization and *ex - vivo* study

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Abstract

Objective: Glibenclamide is important and popular drug required in the treatment of hyperglycemia in Non-Insulin Dependent Diabetes Mellitus (NIDDM). Glibenclamide is affected by first pass metabolism and has a plasma half-life of 4 to 6 hrs, thus require high and frequent administration which in turn results in several side effect like nausea, vomiting, heartburn etc. To overcome this drawback glibenclamide was loaded into ethosomes to improve its therapeutic efficacy and decrease side effect via transdermal route. **Material and methods:** Six different ethosomal formulations with different concentration of ethanol, soya lecithin and cholesterol were prepared by cold method and characterized for vesicle size, polydispersity index (PI), zeta potential, vesicle surface morphology and entrapment efficiency (EE). *Ex - vivo* permeation study was conducted using excised abdominal skin of rat as a permeation barrier using franz diffusion cell. **Results:** Formulation GF4 showed 7 fold higher ($p<0.05$) transdermal flux (56.08 ± 1.78 μg/cm^2/hr) than the drug solution (7.64 ± 2.37 μg/cm^2/hr) and 3 fold higher than GF6 formulation (19.46 ± 2.64 μg/cm^2/hr). The high transdermal flux of GF4 formulation may be attributed to its smaller vesicle size (119.4 ± 1.1527 nm) and high entrapment efficiency. Based on the above findings the GF4 ethosomal formulation was converted to ethosomal gel formulation by incorporating it into 1% Carbopol 324 gel, which was further evaluated and compared with conventional gel formulation for skin permeability across the excised rat skin. **Conclusion:** Ethosomal gel formulation show significantly high ($p<0.05$) transdermal flux (37.23 ± 1.24 μg/cm^2/hr) as compared to conventional gel formulation (16.35 ± 1.75 μg/cm^2/hr). It can be concluded that ethosomal gel formulations is an effective tool for transdermal delivery of glibenclamide.

Keywords: Lecithin, Glibenclamide, ethosome, ethosomal gel, Non-Insulin Dependent Diabetes Mellitus

Introduction

Since skin offers an excellent barrier to molecular transport, except for highly lipophilic, low molecular weight drugs, to overcome this difficulty lipid vesicle are use in delivery system (Ambekar et al., 2011; Pathan et al., 2016). In the past decades, topical delivery of drug by lipid vesicle formulation has evoked considerable interest. One of the major advances in vesicle research is the finding that some specially designed vesicles possessed properties that allowed them to successfully deliver drugs in deeper layer of skin (Sheo et al., 2010). Touitou et al. (2000) has introduced ethosome with enhanced skin permeability as compared to conventional liposomes (Touitou et al., 2000). Higher concentration of ethanol is responsible for increased penetration of the loaded drug across the skin with subsequent high transdermal flux (Inayat et al., 2018). Ethosome contain relatively high concentration of ethanol (up to 50%) in comparison to liposome along with cholesterol and phospholipids (Ibrahim et al., 2016). Steady state drug concentration through out the treatment is required in diseases which are chronic in nature like diabetics; hypertension etc. Transdermal route is the most suitable route for delivery of such drugs (Pandya et al., 2011).

Glibenclamide an oral hypoglycemic agent commonly

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use in treatment of Non-Insulin Dependent Diabetes Mellitus (NIDDM) on oral administration is associated with several side effects like hypoglycemia, frequent GI side effects such as nausea, vomiting, heartburn, anorexia, increased appetite etc. and may even cause hyper-insulinemia because of inter individual variations (Vidhi et al., 2014). Transdermal drug delivery system provides input of such drug directly into the blood with sustain release of drug across the skin at programmed and controlled rate, thus reducing the side effect associated with oral therapy (Sridevi et al., 2000; Rajesh et al., 2013; Prabhu et al., 2014). The main aim of the present study is to develop novel glibenclamide loaded ethosomal gel formulation with enhanced permeability across the skin.

Materials and methods

Materials

Glibenclamide was obtained as a gift sample from USV Laboratories, Ratinagari; Maharashtra. Soya lecithin 30% was purchased from Himedia Laboratories, Pvt. Ltd., Mumbai, India. Ethanol, propylene glycol, cholesterol, carbopol 934, methyl paraben was purchased from SD Fine chemicals Ltd. Mumbai, India. All the chemicals used were of analytical grade and double distilled water was used throughout the study. Due permission was obtained from Institution Animal Ethics Committee of Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Ahmednagar (Reg. No: 1670/PO/ReBiBt/S/12/ CPCSEA) to conduct the experiment.

Preparation of ethosomes

The ethosomes was prepared by cold method. Glibenclamide was dissolve in different concentrations of ethanol and water in required quantity along with 10 ml of propylene glycol by maintaining the concentration of soya lecithin. Required quantity of ethanolic mixture was taken separately and heated at 30 ^{0}C on a water bath; water was slowly added to the above mixture drop wise in the center of the vessel. The resulting mixture was stirred at 700 rpm for 10 min to obtain the ethosomal vesicles. Ethosomes thus obtained were subjected to sonication at 4 ^{0}C and stirring at 700 rpm for 15 min (3 cycles at a gap of 5 min) (Pathan et al., 2016b). The compositions of all the formulations are shown in table 1.

Preparation of conventional and ethosomal gel

For the preparation of ethosomal gel and conventional gel, carbopol 934 (1% w/w) was first dispersed in 8 ml ethanol followed by addition of distilled water, 0.2% methyl paraben and 0.02% propyl paraben as preservatives. To this, triethanolamine (1% w/v) was added with continuous stirring (20 min) until a transparent alkaline gel was obtained. The gel base so prepared was added to GF4 ethosomes and drug respectively by continuous stirring which leads to ethosomal gel formulations and conventional gel (Saroha et al., 2013)

Characterization of ethosomes

Vesicles size, polydispersity index (PI) and zeta potential

Vesicle size and zeta potential were determined by dynamic light scattering method (DLS), using a computerized inspection system (Malvern Zetamaster, ZEM 5002, Malvern, UK). The measurement was done in triplicates. The polydispersity index (PI) was used as a measurement of the particle size homogeneity for the prepared ethosomal

Table 1. Composition of different ethosomal formulations

Formulation Code	% Drug	% Soya Lecithin	% Cholesterol	% Ethanol	% Propylene glycol	Distilled Water
GF1	1	1	2	30	10	Q.S.
GF2	1	1	1	35	10	Q.S.
GF3	1	1.5	2	30	10	Q.S.
GF4	1	1.5	1	35	10	Q.S.
GF5	1	2	1	30	10	Q.S.
GF6	1	2	2	35	10	Q.S.

Table 2. Composition of conventional and ethosomal gel formulations

Formulation Code	Carbopol 934 (%)	Triethanolamine (%)	Methyl paraben (%)	Propyl paraben (%)
Ethosomal Gel	1	1	0.2	0.02
Conventional Gel	1	1	0.2	0.02

formulations. Polydispersity index (PI) less than 0.4 indicates a homogenous and monodisperse population (Inayat et al., 2018; Touitou et al., 2000).

Transmission electron microscopy (TEM)

The morphology and structure of selected ethosomal formulation was determined by the Transmission Electron Microscopy (Philips CM 200 Electron Microscope) at Indian Institute of Technology Bombay (IIT Bombay) Powai. A drop of the sample was placed onto a carbon-coated copper grid to leave a thin film. Before the film dried on the grid, it was stained with 1% phosphotungstic acid. A drop of the staining solution was added onto the film, and the excess of the solution was drained off with a filter paper. The grid was allowed to air dry thoroughly, and samples were viewed in a TEM (Heeremans et al., 1995).

Percent entrapment efficiency (EE)

The entrapment capacity of ethosomal vesicles was determined by ultracentrifugation method. The drug containing ethosomal formulations were kept overnight at 4 °C and centrifuged in ultracentrifuge (Tarsons) at 12000 rpm for 30 min. The sediment and supernatant was removed and drug amount was determined by using UV-Visible spectrophotometer in both the sediment and the supernatant. The entrapment capacity was calculated by using following equation,

$$[(T\text{-}S)/T] \times 100$$

Where, *T* is the total amount of drug that is detected both in the supernatant and sediment, and S is the amount of drug found in the supernatant (Sujitha et al., 2014).

Evaluation of conventional and ethosomal gel

pH and viscosity measurement

The pH of the conventional and ethosomal gel was determined by using digital pH meter model 111 E (HICON New Delhi India) and viscosity was measured by using Brookfield viscometer R/S-CPS (Brookfield Engineering Lab, Inc, USA) using T-spindle S-93 at 20 rpm (22.36×10^{-3} g). The temperature was maintained at $25\ ^0C \pm 1\ ^0C$ (Dheeraj et al., 2013).

Extrudability

The extrudability test was carried out using hardness tester. A 5 gm of gel was filled into the aluminium collapsible tube. The plunger is subjected to hold the tube properly. The 1 gm/cm^2 force was applied for the 30 secs; the quantity of gel extruded from the tube was measured. The procedure was repeated for three times.

Swelling index

To determine the swelling index of prepared gels 1 gm of gel was taken on porous aluminium foil and then placed separately in a 50 ml beaker containing 10 ml 0.1 N NaOH. Then samples were removed from beaker at different time intervals and kept it on dry place for 30 min and reweighed. Swelling index was calculated as follows.

$$\text{Swelling Index (SW)}\ \% = [(Wt - Wo)/Wo] \times 100$$

Where, (SW) % = equilibrium percent swelling.

Wt = Weight of swollen gel after time t.

Wo = Original weight of gel at zero time.

Drug content

One gram of gel was dissolved in a 100 ml of phosphate buffer pH 6.8 stirred constantly for two hours using magnetic stirrer. The resultant solution was filtered and content was analysed by U.V spectrophotometer.

Spreadability

Spreadability was determined by using modified wooden block and glass slide apparatus. A measured amount of gel (0.1 gm) was placed on fixed glass slide; the movable pan with a glass slide attached to it and was placed over the fixed glass slide, such that the gel was sandwiched between the two glass slides for 5 min. The weight was continuously removed. spreadability was determined using the formula (Nida et al., 2012).

$$S = M/T$$

Where, S is the spreadability in gm/sec,

M is the mass in gm & T is the time in seconds.

Ex - vivo permeability study

Albino rats (Wistar strain), 6 – 8 weeks old, weighing 120 – 150 gm was sacrificed by spinal cord delocalization, tested animals were pre shaved carefully and the abdominal skin was then separated from the underlying connective tissue with the scalpel, the excised skin was then placed on aluminum foil and the dermal side of the skin was gently teased off for any adhering fat or subcutaneous tissue. Prepared skin was then mounted on franz diffusion cell (area, 3.14 cm^2) and the test formulation was applied on the epidermal side of the skin. 1 ml sample was withdrawn from the receptor compartment containing 25 ml of the phosphate buffer pH 7.4 maintained at $37°C \pm 1^0C$, at appropriate time intervals and analyzed spectrophotometrically to determine the cumulative amount of drug permeated across the skin in the receptor medium irrespective of the drug deposited in the skin layers. An equal volume of fresh phosphate buffer pH 7.4 was replaced after each sampling into the receptor compartment. The study was performed in triplicate and average values were calculated. The percent cumulative amount of drug permeated across the skin per square surface area was evaluated and plotted against time to calculate the flux (Benson et al., 2005).

Statistical analysis

The data were statistically evaluated using one-way ANOVA. The significance of the result was analyzed at $P<0.05$.

Results and discussion

Characterization of ethosomes

Vesicle size, polydispersity index, zeta potential and % entrapment efficiency

Prepared ethosomal formulations were optimized based on vesicle size, polydispersity index, zeta potential and % entrapment efficiency. Examination of prepared formulations revealed that average vesicle size of optimized formulation i.e GF4 was 119.4 ± 1.1527 nm. The vesicle mean diameters for all formulations are shown in table 3. Narrow peak for all the formulations, is an indication that the size of vesicle population is comparatively uniform. The largest size of the vehicle was present in preparation containing 35% ethanol, 2% soya lecithin and 2% cholesterol i.e GF6 (510.1 ± 1.0577 nm), while the smallest vehicle size was present in preparation containing 35% ethanol, 1.5% soya lecithin and 1% cholesterol i.e GF4 (119.4 ± 1.1527 nm). Polydispersity index was determined as a measure of homogeneity in formulation (figure 1 and 2). Polydispersity index for GF4 formulation was found to be 0.334 ± 0.020 which is an indication of homogeneous population of ethosome vesicle in formulation.

Drug loading and % entrapment efficiency is considered as key parameters to evaluate the delivery potentiality of any vesicular formulations. The % entrapment efficiency of various ethosome formulations is shown in table 3. Form the results obtained it was observed that entrapment efficiency as determined by centrifugation method was 67 ± 3.2415% for GF4 formulation, containing 35% ethanol and 1.5% lecithin. Amounts of ethanol and lecithin, used for ethosome preparation, were found to have influenced the entrapment efficiency. It can be observed that increasing ethanol concentration from 30% to 35% increases the entrapment efficiency.

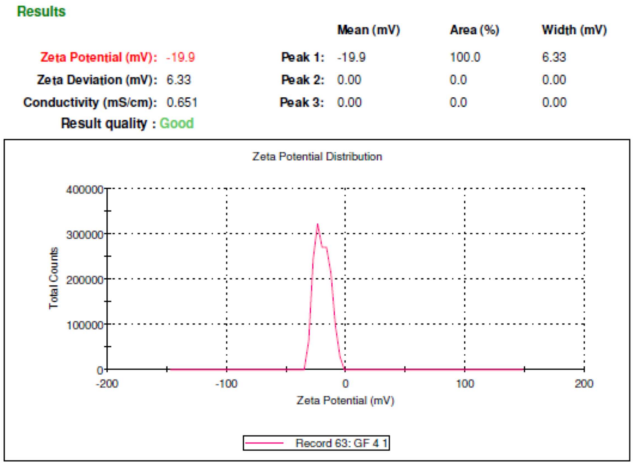

Figure 1. Zeta potential of GF4 ethosomal formulation

Table 3. Characterization of ethosomal and gel formulations

Formulation Code	Vesicle Size (nm)	Polydispersity Index (PI)	% Entrapment efficiency	Zeta Potential (mV)	Flux (µg/cm^2/hr)	Permeability Coefficient (cm/hr)
GF1	168.1 ± 3.5821	0.426 ± 0.057	56 ± 1.4314	-18.7	43.59 ± 1.90	0.02906
GF2	141.0 ± 1.6658	0.451 ± 0.037	61 ± 3.5217	-19.3	47.31 ± 1.54	0.03154
GF3	361.6 ± 1.1732	0.676 ± 0.015	48 ± 1.7315	-20.4	29.11 ± 2.34	0.01941
GF4	119.4 ± 1.1527	0.334 ± 0.020	67 ± 3.2415	-19.9	56.08 ± 1.78	0.03739
GF5	465.2 ± 2.1154	0.765 ± 0.120	41 ± 1.2414	-20.3	22.85 ± 2.54	0.01523
GF6	510.1 ± 1.0577	0.566 ± 0.010	40 ± 2.5217	-21.6	19.46 ± 2.64	0.01297
Drug Solution	--	---	---	---	7.64 ± 2.37	0.00509
Ethosomal Gel	--	--	--	--	37.23 ± 1.24	0.02672
Conventional Gel	--	--	--	--	16.35 ± 1.75	0.01155

Values are expressed in mean ± SD, Where n = 3

Table 4. Different evaluation parameters of ethosomal gel and conventional gel formulations

Formulation Code	pH	Viscosity (cp)	Swelling index (%)	Wt. extruded from tube (gm/cm^2)	Drug content (%)	Spreadability (g.cm/sec)
Ethosomal Gel	6.15 ± 0.621	35500 ± 5.760	10 ± 1.357	0.59 ± 0.421	91.05 ± 3.028	5.4 ± 0.676
Conventional Gel	6.73 ± 0.354	41800 ± 6.756	12 ± 1.257	0.61 ± 0.623	88.14 ± 2.546	5.91 ± 1.852

Values are expressed in mean ± SD, where n = 3.

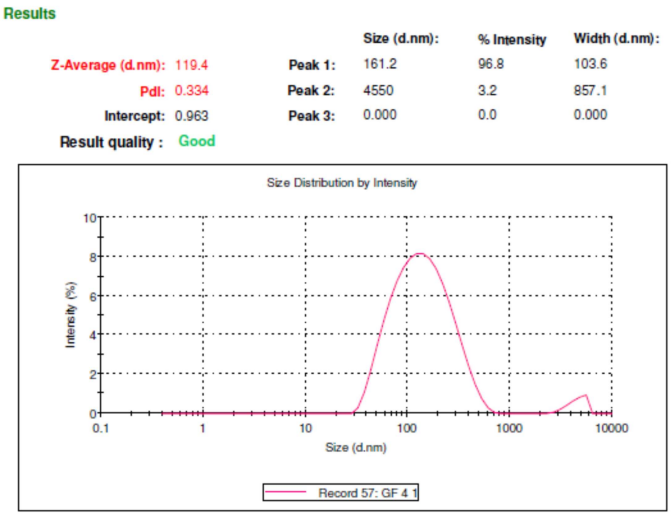

Figure 2. Vesicle size analysis of GF4 ethosomal formulation

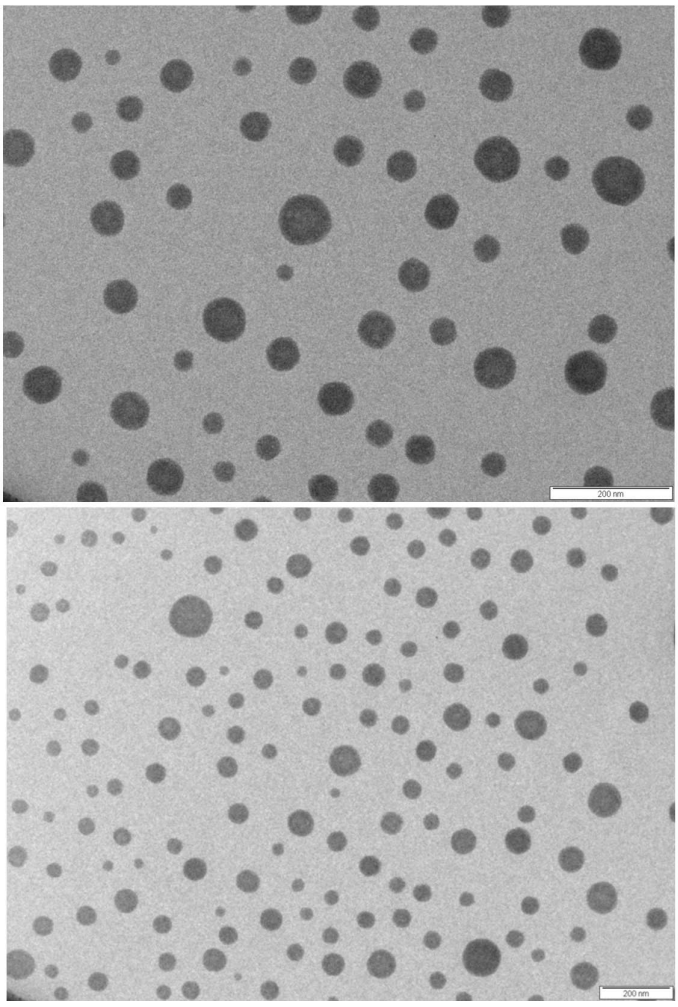

Figure 3. Transmission electron microscopy images of ethosomal GF4 formulation

Transmission electron microscopy (TEM)

The results of transmission electron microscopy showed that the prepared ethosome formulations are uniform in size and spherical in shape (figure 3).

Evaluation of conventional and ethosomal gel

Physical evaluation/appearance

All the formulations were transparent, smooth and homogeneous.

pH, rheology, extrudability, swelling index and drug content determination

All the formulations were evaluated for pH, viscosity, swelling index, extrudability and drug content. The results are shown in table 4.

Ex – vivo permeation study

Ex - vivo skin permeation study for ethosomal formulation was carried out for 7 hrs using excised rat skin and compared with ethanolic solution of drug. The release data for the flux for all formulation is recorded in table 3 and was found to be maximum for GF4 formulation (56.08 ± 1.78 µg/cm^2/hr) as compared to other formulations. High permeation flux for GF4 formulation may be due to high concentration of ethanol, high entrapment efficiency and smaller vesicle size. The permeation profile of all the ethosomal formulations through excised rat skin is shown in figure 4. The flux values for all the formulations are shown in table 3. The flux for all the ethosomal formulation range from 19.46 ± 2.64 µg/cm^2/hr to 56.08 ± 1.78 µg/cm^2/hr. Flux for GF4 ethosomal formulation (56.08 ± 1.78 µg/cm^2/hr) was found to be 3 fold higher than that of GF6 formulation (19.46 ± 2.64 µg/cm^2/hr) which was found to be significant ($p<0.05$) and 7 fold higher than that of the ethanolic solution of drug (7.64 ± 2.37 µg/cm^2/hr). This may be due to the small vesicle size, high entrapment efficiency and high concentration of ethanol along with the synergistic effect of phospholipids and ethanol in vesicle formulations. GF4 ethosomal formulation is considered as the best and optimized formulation from vesicle size distribution, polydispersity index, zeta potential, drug entrapment efficiency and ex - *vivo* permeation study results. Hence GF4 ethosomal formulation was considered for formulating

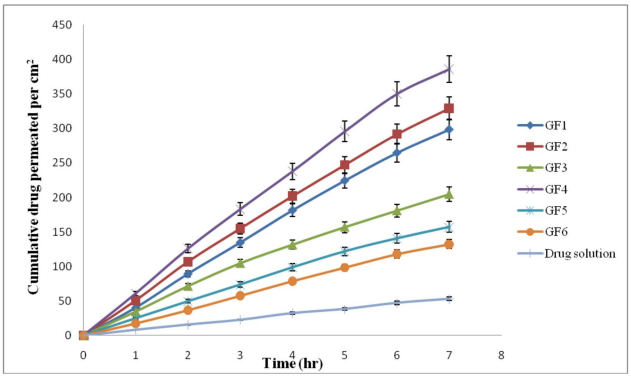

Figure 4. Cumulative drug permeated per cm^2 of ethosome formulations and drug solution

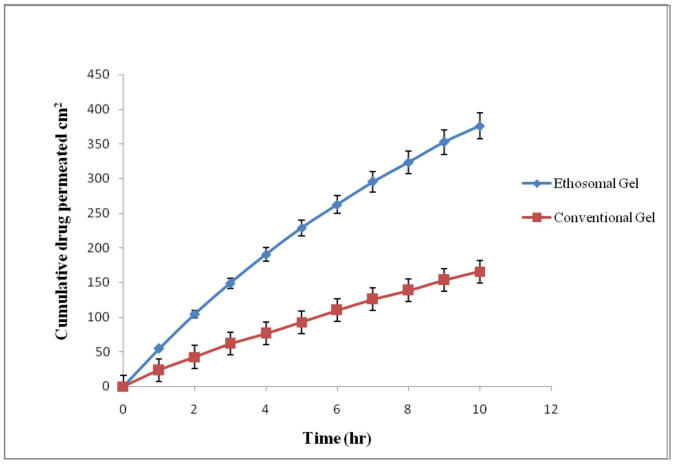

Figure 5. Cumulative drug permeated per cm^2 of ethosomal gel and conventional gel

into gel. The ethosomal gel formulation so obtained was evaluated for skin permeability for 10 hr, the transdermal flux value for ethosomal gel formulation (37.23 ± 1.24µg/cm^2/hr) was 2 folds higher ($p<0.05$) when compared to the conventional gel formulation (16.35 ± 1.75µg/cm^2/hr).

Conclusion

The glibenclamide loaded ethosome formulation was formulated and characterized for vesicle size, zeta potential, Polydispersity Index (PI), entrapment efficiency and *ex - vivo* permeability across excised rat skin. GF4 formulation showed smallest vesicle size (119.4 ± 1.1527 nm), PI (0.334 ± 0.020) and highest % entrapment efficiency (67 ± 3.2415%). *Ex - vivo* permeation study result for GF4 formulation shows significant higher ($p<0.05$) transdermal flux across excised rat skin as compared to other formulations. The optimized ethosomal formulation i.e GF4 was formulated into ethosomal gel formulation by incorporating it into 1% w/w carbapol 934 gel. The transdermal flux of ethosomal gel formulation was calculated and compared with conventional gel formulation. The result showed that ethosomal gel formulation shows significant ($p<0.05$) higher permeability as compared to conventional gel formulation. Finally, it can be concluded that formulated ethosome gel formulation loaded with glibenclamide can be prepared with appropriate size, maximum drug entrapment efficiency and enhanced transdermal flux as compare to conventional gel formulation. The efficient skin permeability makes ethosomal gel formulation a potential and effective transdermal drug delivery system for glibenclamide.

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Conflict of interest: Nil

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