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DEVELOPMENT, CHARACTERIZATION AND *IN-VITRO* EVALUATION OF ETHOSOMAL GEL FOR TRANSDERMAL DELIVERY OF SAXAGLIPTIN

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ABSTRACT

The aim of the present work was to formulate, characterize and evaluate ethosomal gel formulation for transdermal delivery of saxagliptin. Six different ethosomal formulations with different concentration of ethanol, soya lecithin and cholesterol were prepared using cold method and characterized for vesicle size, polydispersity index (PI), zeta potential, vesicle morphology and entrapment efficiency (EE). The ethosomal formulation F6 showed smallest vesicle size of 117.3 ± 1.0577 nm with homogeneous population of ethosomes indicated by polydispersity index (PI) of 0.230 ± 0.010 and highest entrapment efficiency i.e 64 ± 1.5329 %. Based on the transmission electron microscopy (TEM) analysis all the ethosomal formulations were found to be spherical in shape. *In vitro* skin permeation study across excised rat skin showed highest transdermal flux for F6 formulation (51.98 ± 2.051 µg/cm^2/hr) i.e 7 fold higher as compared to ethanolic solution of drug (7.04 ± 0.911 µg/cm^2/hr) and 4 fold higher when compared to F1 formulation (12.37 ± 1.733 µg/cm^2/hr) in 7 hrs. The results showed that increase in concentration of ethanol decreases vesicle size with increased skin permeability. Optimized ethosome formulation F6 was incorporated into 1% carbopol 934 to get ethosomal gel and evaluated for skin permeability across excised rat skin using franz diffusion cell (area, 3.14 cm^2) and was compared with conventional gel. Results indicate that the developed ethosomal gel formulation is potential, safe and effective transdermal drug delivery system for saxagliptin through skin.

INTRODUCTION:

Throughout the past two decades, the transdermal drug delivery system has become a proven technology that offers a variety of significant clinical benefits over other dosage forms as it avoids hepatic first pass metabolism, improves patient compliance and minimizes harmful side effects of a drug[1, 2, 3]. The transdermal route is ideally suitable for drugs that need to be administered for diseases those are chronic in nature and required a steady state drug concentration throughout the treatment[4]. Transdermal drug delivery offers many important advantage, for instance, it is easy and painless, it protects the active compound from gastric

enzymes, controls absorption rate, variations in delivery rates, interference due to the presence of food, suitable for unconscious patients and enables fast termination of drug delivery, if needed[5]. Various novel approaches in drug delivery technology have been made over many years; one such technique is vesicular drug delivery systems. Significant enhanced delivery of drug through transdermal route could be obtained by using vesicular drug carrier systems like liposomes and ethosomes[6]. Ethosomal systems were found to be significantly superior at delivering drugs through the skin in terms of both quantity and depth that are capable of delivering various chemical applications [7].

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose concentration hyperglycemia caused by insulin deficiency, often combined with insulin resistance [8]. Despite of availability of large varieties of hypoglycemic agents, the treatment of type II diabetes remains elusive[9]. Saxagliptin, is an oral hypoglycemic of the dipeptidyl peptidase – 4 (DPP - 4) inhibitor class of drug used in the treatment of non-insulin-dependent diabetes mellitus, has been associated with severe and sometimes fatal hypoglycemic reactions after oral therapy because of higher inter-individual variations [10]. Since these drugs are usually intended to be taken for a long period, patient compliance is also very important, hence the objective of present study involve formulation of ethosomal gel using soya licithin as vehicle forming agent, ethanol, propylene glycol and cholesterol[11]. In the present study ethosomes with different concentration of phospholipids and ethanol were prepared and evaluated for particle size, polydispersity index (PDI), zeta potential, extrudability and entrapment efficiency (EE) then a chosen formula was incorporated into a gel dosage form to investigate the potential application of ethosomal gel for transdermal delivery of saxagliptin an anti diabetic drug.

Material and Method:

Material: Saxagliptin was obtained as a gift sample from MSN Laboratories, Hyderabad. Soya lecithin 30% was purchased from Himedia Laboratories, Pvt. Ltd., Mumbai, India. Ethanol, propylene glycol, cholesterol, carbapol 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.

METHOD:

Preparation of ethosome: The ethosome was prepared by cold method. The ethosomal formulations were prepared by dissolving saxagliptin in different concentrations of ethanol and water in required quantity along with 10 ml of propylene glycol by maintaining the concentration of soya lecithin. The ethanolic mixture in required quantity was taken separately and heated at 30 ^{0}C on a water bath. The distilled water was slowly added to ethanol 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) [12]. The compositions of all the formulations are shown in Table no 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 F6 ethosome formulation and drug respectively by continuous stirring which leads to ethosomal gel base formulation and conventional gel formulation [13.] (Table no: 02)

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 formulation were diluted by 1/4th distilled water before measurement and measured three times at a scattering angle of 90^0 between laser and detector. The polydispersity index (PI) was used as a measurement of the width of the size distribution. Polydispersity index (PI) less than 0.4 indicates a homogenous and monodisperse population. Zeta potential was measured as the particle electrophoretic mobility means of laser microelectrophoresis in a thermostated cell.

Transmission electron microscopy (TEM): The morphology of selected ethosomal formulation was evaluated by the TEM (Philips CM 200 Electron Microscope) at Indian Institute of Technology Bombay (IIT Bombay) Powai, at an acceleration voltage of 20 kV being used to visualize ethosomes. 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 negatively 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. TEM images of ethosomes were taken which are presented in Figure 01

% Entrapment efficiency (EE): The entrapment capacity of saxagliptin by ethosomal vesicles was determined by ultracentrifugation. The drug containing ethosomal formulations were kept overnight at 4°C and centrifuged in ultracentrifuge (Tarsons) at 12000 rpm for 30 min. The supernatant was removed and drug amount was determined by using UV-Visible spectrophotometer at 210 nm in both the sediment and the supernatant. The entrapment capacity was calculated as follows, [(*T*-S) /*T*] *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[14].

Evaluation of conventional and ethosomal gel:

pH and viscosity measurement: The pH of the 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°C± 1°C[15].

Extrudability: The extrudability test was carried out using hardness tester. A 5 gm of gel was filled into the aluminium collapsible tubes. The plunger is subjected to hold the tube properly. The $1gm/cm^2$ force was applied for the 30 sec; 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 ethosomal gel 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.

Swelling Index (SW) % = [(Wt – Wo) / Wo] × 100

Where, (SW) % = equilibrium percent swelling.

Wt = Weight of swollen ethosomal gel after time t.

Wo = Original weight of ethosomal gel at zero time.

Drug content: 1 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 of gel was determined by modified wooden block and glass slide apparatus. A measured amount of gel (0.1 g) 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[16].

S = M/T Where, S is the spreadability in g/s, M is the mass in grams & T is the time in seconds.

***In vitro* skin permeability study:**

Albino rats (Wistar strain), 6–8 weeks old, weighing 120–150 g were sacrificed by spinal cord delocalization. For the *in vitro* permeability study, 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 ± 1°C, at appropriate time intervals and analyzed spectrophotometrically at 210 nm 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 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 [17].

RESULT AND DISCUSSION:

Characterization of ethosomes:

Vesicle size,polydispersity Index, zeta potential and % entrapment efficiency: Ethosome formulations were evaluated for vesicle size, polydispersity index, zeta potential and % entrapment efficiency. The vesicle mean diameters for all formulations are shown in table no 3.The result shows narrow peak for all formulations, which indicating that the size of vesicle population is comparatively uniform. In accordance with other researcher, this decrease in the mean diameter of the vesicle is due to the presence of ethanol [18]. Higher concentration of ethanol produced smaller vesicle size. Ethanol confers a surface negative net charge to the ethosome which causes the size of vesicles to decrease[19]. The size of ethosomal vesicles increase with decreasing ethanol concentration. It was observed that the vesicles of composite ethosomal formulations (F1 – F6) varied in size range of 766.3 ± 3.5821 nm to 117.3 ± 1.0577 nm. Polydispersity index was determined as a measure of homogeneity in formulation (see Figure no 1 and 2). Polydispersity index indicate homogeneous population of ethosome vesicle in formulation, it was found to be 0.230 ± 0.010 for F6 formulation. Drug loading and % entrapment efficiency are key parameters that evaluate the delivery potentiality of a system. The % entrapment efficiency of various ethosome formulations is shown in Table no 3. Amounts of ethanol and lecithin, used for ethosome preparation, were found to have influenced the entrapment efficiency. % entrapment efficiency of ethosome formulation was found in the range of 64 ± 3.5329 % to 40 ± 1.3605 % highest for F6 formulation.

Table No: 1: Composition of different ethosomal formulations

Formulation Code	% Drug	% Soya Lecithin	% Cholesterol	% Ethanol	% Propylene glycol	Distilled Water
F1	1	1	2	30	10	Q.S.
F2	1	1	1	35	10	Q.S.
F3	1	1.5	2	30	10	Q.S.
F4	1	1.5	1	35	10	Q.S.
F5	1	2	1	30	10	Q.S.
F6	1	2	2	35	10	Q.S.

Table No: 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

Table no 3: Characterization of ethosomal and gel formulations.

Formulation Code	Vesicle Size (nm)	Polydispersity Index (PI)	% Entrapment efficiency	Zeta Potential (mV)	Flux ($\mu g/cm^2/hr$)	Permeability Coefficient (cm/hr)
F1	766.3 ± 3.5821	0.620 ± 0.057	49 ± 2.5507	-19.1	12.37 ± 1.733	0.00824
F2	321.8 ± 1.6658	0.745 ± 0.037	60 ± 3.9539	-24.5	18.60 ± 1.77	0.01240
F3	139.2 ± 1.1732	0.462 ± 0.015	47 ± 1.4509	-19.0	29.57 ± 2.51	0.01971
F4	140.3 ± 1.1527	0.405 ± 0.020	62 ± 2.1527	-17.4	28.37 ± 1.66	0.01891
F5	240.5 ± 2.1154	0.471 ± 0.120	40 ± 1.3605	-18.6	25.18 ± 1.63	0.01678
F6	117.3 ± 1.0577	0.230 ± 0.010	64 ± 3.5329	-17.5	51.98 ± 2.051	0.03465
Drug Solution	--	---	---	---	7.04 ± 0.911	0.00469
Ethosomal Gel	--	--	--	--	34.33 ± 1.58	0.02288
Conventional Gel	--	--	--	--	13.81 ± 1.25	0.00920

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

Table no 4: pH, viscosity, swelling index, wt. extruded from tube and drug content 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	6.45 ± 0.521	36400 ± 3.560	11 ± 1.253	0.64 ± 0.485	92.03 ± 2.123	5.8 ± 0.896
Conventional Gel	6.23 ± 0.234	42700 ± 5.891	13 ± 1.789	0.62 ± 0.783	87.12 ± 2.896	6.01 ± 1.235

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

Figure no 1: Zeta potential of F6 ethosomal formulation.

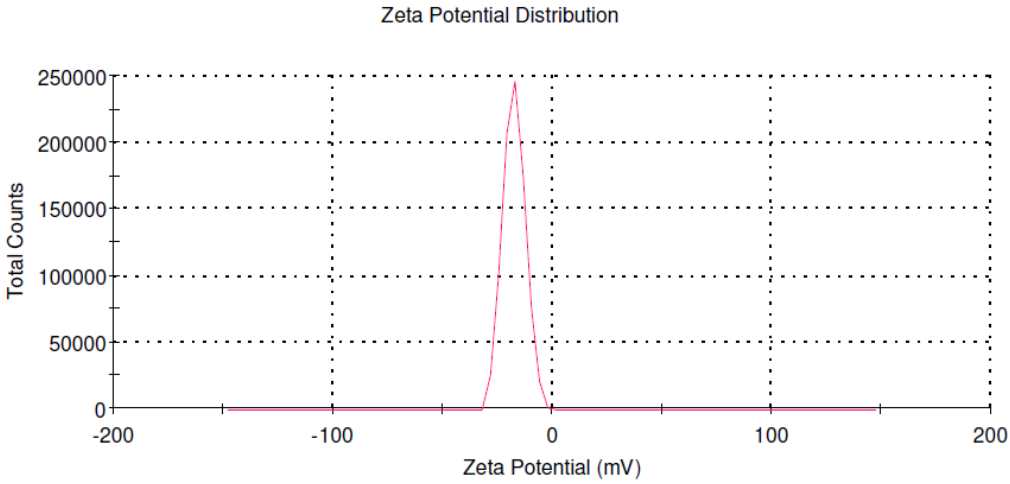


Figure no 2: Vesicle size analysis of F6 ethosomal formulation.

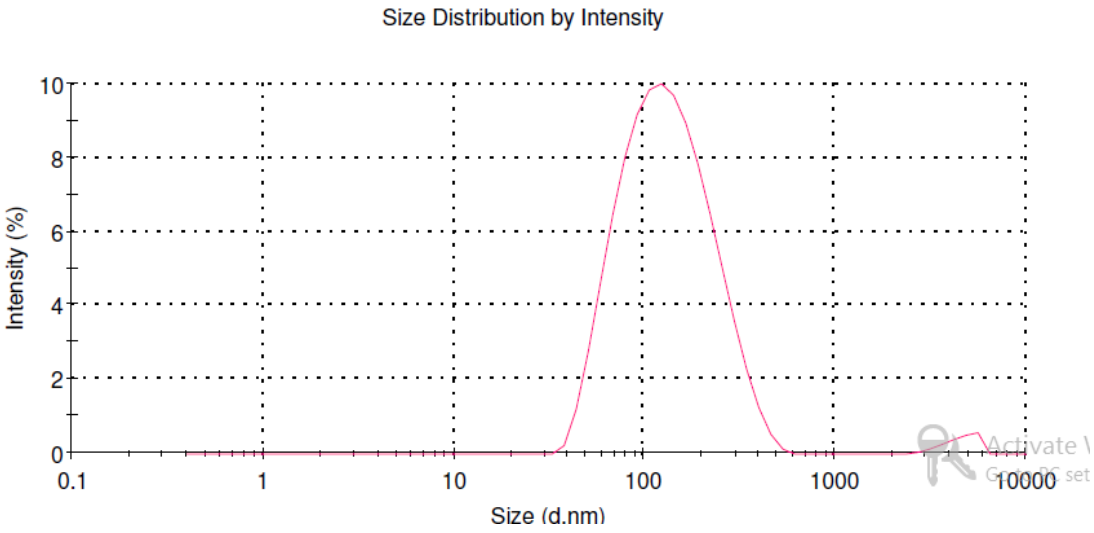


Figure no 3: Transmission electron microscopy image of ethosomal F6 formulation

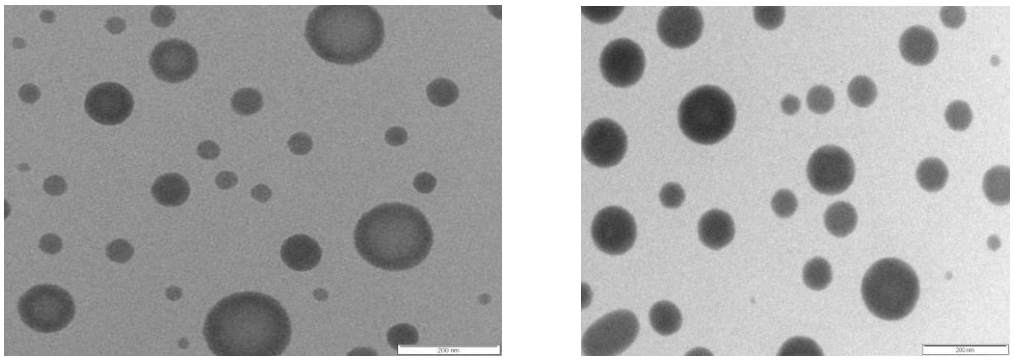


Figure no 04: Cumulative drug permeated per cm^2 of ethosome formulations and drug solution

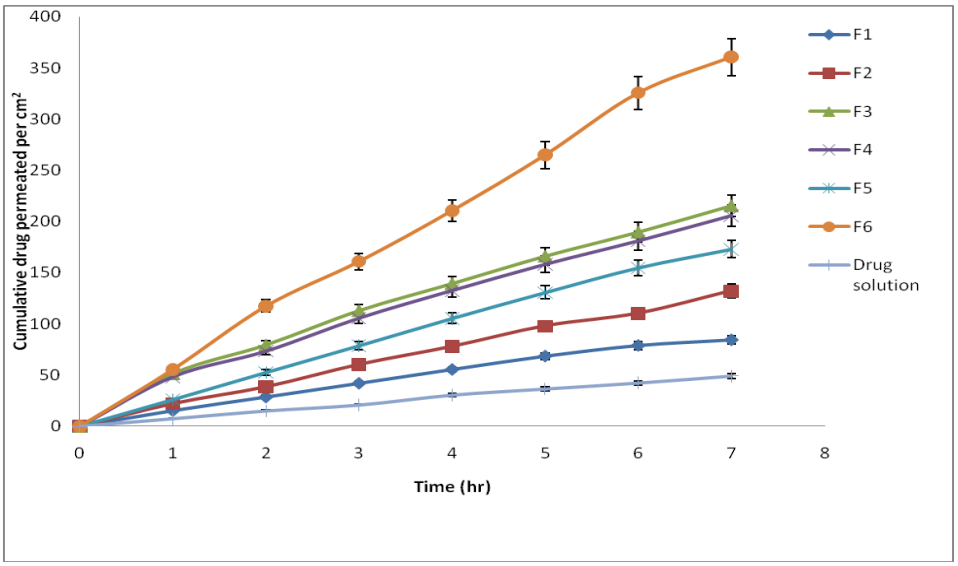
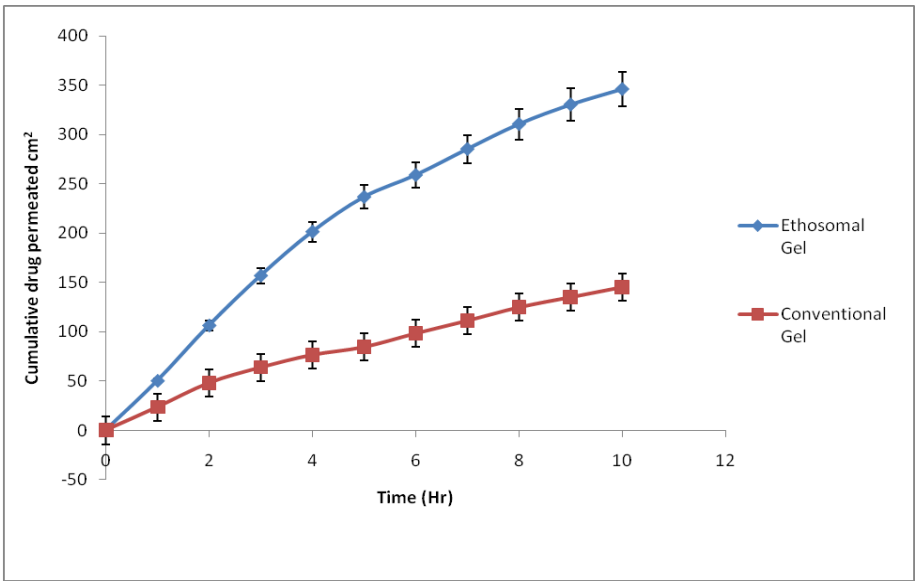


Figure no 05: Cumulative drug permeated per cm^2 of ethosomal gel and conventional gel



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 no 03)

Evaluation of 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 no 4.

***In vitro* skin permeation study of gel formulation:**

In vitro skin permeation study for ethosomal formulation was performed for 7 hrs using excised rat skin and compared with ethanolic solution of drug. Transdermal flux for different ethosomal formulation ranged between 12.37 ± 1.733 to 51.98 ± 2.051 µg/cm^2/hr (see table no 3). Flux for F6 formulation was 4 fold higher

than that of F1 formulation (12.37 ± 1.733 µg/cm^2/hr) and 7 fold higher than that of the ethanolic solution of drug (7.04 ± 0.911µg/cm^2/hr). This may be due to the small particle size, high entrapment efficiency and high concentration of ethanol. F6 formulation is considered as the best and optimized formulation from particle size distribution, polydispersity index, zeta potential, drug entrapment efficiency and *in vitro* permeation study results. Hence it was considered for formulating into gel. The ethosomal gel was evaluated for skin permeability for 10 hrs, the transdermal flux value for ethasomal gel (34.33 ± 1.58 µg/cm^2/hr) was significantly higher when compared to the conventional gel (13.81 ± 1.25 µg/cm^2/hr).

CONCLUSION:

Ethosome loaded with saxagliptin can be prepared with appropriate size and maximum drug entrapment efficiency. F6 formulation showed highest transdermal flux across excised rat skin was composed of 2% soya lecithin and 35 % of ethanol. The best ethosomal formulation was formulated into ethosomal gel and transdermal flux was calculated and compared with conventional gel. From the results it can be concluded that ethosomal gel formulation is potential and effective transdermal drug delivery system for saxagliptin.

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