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Research Article

Formulation and Evaluation of Vildagliptin Ethosomal Gel for Diabetes Mellitus

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ABSTRACT

The main aim of the present investigation was formulation and evaluation of Vildagliptin ethosomal gel for diabetes mellitus. The ethosomes of Vildagliptin was prepared by cold method. Total nine formulation (F1 – F9) of ethosomes was prepared with different concentration of phospholipid (1,2,3%w/w) and ethanol (25,30,35%w/v). They were evaluated for vesicular shape, size, entrapment efficiency, drug content, In-vitro studies, Ex-vivo studies and stability studies. Drug-excipient compatibility studies showed that there is no interaction between the drug and excipients. The formulation F6 was selected as the best formulation (ethanol 35%w/v and phospholipid 3%w/w) due its optimum vesicle size, maximum entrapment efficiency of 58.92% and drug content of 95.43± 0.16. F6 showed In-vitro drug release of 92.06%. It was further incorporated into gel by using carbopol 934 (1, 1.5, 2% w/w) as a base. The carbopol concentration of 1.5% w/w gives the maximum In-vitro release of 94.34% and showed Ex-vivo drug release of 77.46% on rat skin. The stability studies were performed on F6 formulation at two different temperatures of 25±2ºC/60%±5% RH and 4±2ºC/40%RH for the period of 3 months shows no significant changes were found. These results confirmed ethosomal gel as the potential candidate for transdermal delivery of Vildagliptin for the treatment of diabetes mellitus.

Keywords: TDDS, Vildagliptin, Ethosomes, Diabetes mellitus, Lecithin.

INTRODUCTION

DM or diabetes mellitus, is also referred to as sugar. Diabetes mellitus is a common endocrine illness that results from either the body's cells not properly responding to the body's insulin levels or the pancreas's inability to produce enough insulin as a result of the loss of beta (β) cells. When the body's glucose levels are high, the β-cells generated by the pancreas create insulin to decrease blood glucose level and α-cells produces glucagon which increases glucose level by promoting the creation of glucose. DM is primarily divided into three categories: Type-I DM, Type-II DM, and gestational DM1.

Diet, exercise, insulin therapy, and medications such biguanides, sulfonylureas, alpha- glucoside inhibitors, meglitinidies, thiazolidinediones, and dipeptidyl peptidase inhibitors (DPP-IV) can all be used to manage diabetes mellitus. Vildagliptin is an antihyperglycemic medication that can be taken orally and is used to treat type II diabetes. Vildagliptin slows the breakdown of GLP-1 (Glucagon like peptide-1) and GLP (Glucose dependent insulinotropic polypeptide) by blocking the dipeptidyl peptidase-IV enzyme. As a result, there is an increase in GLP-1 and GIP, which leads to better glycemic control2.

Ethosomes are novel drug delivery systems composed of phospholipids, highly concentrated ethanol, and water. These are flexible, malleable vesicles, and the presence of high concentrations of ethanol disrupts the organization of the skin's lipid bilayer, thus increasing drug penetration through the skin and reducing membrane stiffness. and increase liquidity. Ethosomes can be produced mainly by cold process, hot process and conventional mechanical dispersion process3

The main objective of this research work was to formulate and evaluate Vildagliptin ethosomes by using cold method with varying phospholipid and ethanol concentrations to enhance drug penetration through the skin and maximize drug delivery to appropriate sites and to achieve the desired pharmacological effect. It reduces drug-related side effects, controls drug release over an extended period of time, and facilitates administration.

MATERIALS AND METHODS

MATERIALS

Vildagliptin was collected as a gift sample from Micro Labs Private Limited, Bangalore. Soy lecithin was obtained as a gift sample from VitaeGen Life Sciences, Bangalore. Ethanol,Cholesterol, Propylene Glycol, Triethanolamine, Carbopol 934 are S.D. Fine Chemicals. Pvt. Ltd., Mumbai, India. All chemicals and solvents used were of analytical grade.

METHODS

Preformulation studies

Solubility: Solubility of selected drugs was determined using standard methods using different solvents such as ethanol, dimethylformamide, 0.1N NaOH, phosphate buffer pH 7.4, and n- butanol.

Melting point determination: The melting point of Vildagliptin was determined using Thiel's tube method, in which the drug was placed in a capillary tube, attached to a thermometer, and immersed in a tube containing liquid paraffin. Start heating and

record the temperature at which the drug begins to dissolve.

Determination of λ max and calibration curve: The λ max of Vildagliptin was determined using a UV spectrophotometer by using 0.1N NaOH in wavelength range of 400-200nm and calibration curve was determined by using phosphate buffer pH 7.4 in the wavelength range of 400-200 nm.

Fourier Transform Infrared Radiation (FT-IR): FT-IR studies were performed with drug alone and with other excipients to find compatibility between drug, soyalecithin, and cholesterol used to formulate ethosomal gels. A Tensor 27 instrument was used for studies using the KBr pellet method4.

Preparation of Vildagliptin ethosomes: Ethosomes of Vildagliptin was prepared by using cold method. The drug, soyalecithin and cholesterol were dissolved in ethanol in a covered vessel at room temperature by vigorous stirring using a mixer. Propylene glycol was added during stirring. The mixture was heated to 30°C in a water bath. Water heated to 30° C in a separate vessel was added to the mixture and stirred for an additional 30 minutes in a covered vessel. The vesicle size of the ethosomes preparation was reduced to the desired extent by sonication. Finally, the formulation was stored in the refrigerator5.

Preparation of Vildagliptin ethosomal gel: Ethosomal formulations that showed the best release profile was further incorporated into Carbopol gels. Fixed amount of Carbopol-934 (1%, 1.5%, and 2% w/w) were added to a minimal amount of distilled water for 3 hours. The mixture was then stirred using a mechanical stirrer at 100 rpm to form a homogeneous viscous solution. And ethosomal preparation was added to this. Triethanolamine was added dropwise to neutralize the mixture. Mixing was continued until uniform ethosomal gel was obtained4.

Evaluation of Vildagliptin ethosomes

Determination of pH: A digital pH meter was used to measure the pH of the prepared ethosomal gels and the triplicate measurements were averaged.

Rheological studies: With the aid of a Brookfield viscometer, the viscosity of ethosomal gels was measured. A 30g sample of the gel was taken, and the dial reading was recorded after dipping the spindle grooves into the formulation and starting the motor at various rpms.

Spreadability coefficient studies: Two slides were used, with the top slide pressed with a weight of about 20g after the upper slide had been placed on the lower slide with 0.1g ethosomal gel. The length of time it took for the slides to emerge from the gel was then recorded.

Spreadability = Weight tied on upper slide (g) x Length of glass slide (cm)/ time in sec SEM Analysis: Phosphate buffer (pH 7.4) was used to dilute 0.2 gm of ethosomal gel. The vesicles were sputter coated with gold/palladium (Au/Pd) using a vacuum evaporator and analyzed using Scanning Electron Microscopy after being placed on aluminium stubs with double-sided sticky carbon tape.

Turbidity measurement: The ethosomal formulations were transferred to a 50 ml glass cuvette. With milipore water, 500 NTU (Nephalometric Turbidity Units) were set and a zero reading was established. The cuvette was placed inside the Digital Nephalo Turbidity Meter

132. Make a note of the readings that were shown on the screen and expressed as NTU.

Vesicle size: The ethosomal gel (100mg) was hydrated by adding saline solution (0.9% solution) and occasionally shaking the vial for 10 minutes. Under an optical microscope with a 100x magnification, the dispersion was seen. A calibrated ocular and stage micrometer installed in the optical microscope was used to measure the vesicle sizes.

Drug Entrapment Efficiency: Ethosomal gels were placed into the centrifuge after being diluted with phosphate buffer (pH 7.4). Vildagliptin containing ethosomes were isolated from unentrapped drug by centrifuging at 10,000 rpm for 30 minutes. A sample of supernatant was collected, diluted with phosphate buffer 7.4, and measured at 216nm using a UV spectrophotometer.

$$\% EE = [(Ct - Cf)/ Ct] \times 100$$

Where, Ct is the concentration of total drug and Cf is the concentration of free drug.

Drug Content: The ethosomal gel was weighed out and then dissolved in phosphate buffer (PBS) 7.4 and diluted to 100ml with the same solution (Stock-1). PBS 7.4 was used to dilute a 1ml solution (from stock 1) to a final volume of 10ml. The solution was then filtered, and the drug solution was collected for spectrophotometric analysis at 216nm.

In-vitro diffusion studies: Franz diffusion cells were used to conduct In-vitro diffusion studies. Ethosomal gel was kept on the membrane mounted on donor compartment and phosphate buffer pH 7.4 was filled inside the receptor compartment. The receptor compartment was constantly stirred at 100rpm while the temperature was kept at 37± 0.5ºC. At different time intervals, 1ml sample was withdrawn from receptor compartment and

immediately replaced with fresh buffer solution. After that, samples were spectrophotometrically examined at 216nm6.

Ex-vivo studies

Studies on Ex-vivo skin permeability was performed using the abdomen skin of adult albino rats (10 -12 weeks, weighing 200-250g). The abdominal skin was first freed from hair, followed by the delicate removal of connective and fat tissues, followed by a standard saline wash and drying between two filter papers. Following that, ethosomal gel was placed into the skin which was mounted on donor compartment. Phosphate buffer pH 7.4 was placed in the receptor compartment. At different times, samples were taken out and replaced with newly made buffer solution. The samples were then subjected to spectrophotometric analysis.[7]

Drug release kinetics

Considering a variety of kinetic models, such as the zero order, first order, Higuchi, and Korsmeyer-Peppas models, the kinetics of drug release in several formulations was studied. We can determine which model the drug release complies to by looking at the graphs that were plotted using the regression coefficient (r2) values for Zero-order as% drug released v/s time, First order as log % drug retained v/s time, Higuchi as % drug released v/s √time, and Korsmeyer-Peppas as log% drug released v/s log time8.

Stability studies

The ability of vesicles to retain the drug was assessed by keeping the ethosomal formulation at different temperature. The stability studies were done on most satisfactory formulation at two different temperature i.e. refrigerated temperature (4±2°C/40%RH) and at room temperature (25±2°C/60%±5% RH) for 3 months9.

RESULTS AND DISCUSSION

Vildagliptin was found to be freely soluble in water and ethanol, very soluble in dimethyl formamide, soluble in phosphate buffer (pH 7.4) and 0.1 N NaOH. Slightly soluble in n-Butanol. Melting point of Vildagliptin was found to be 153°C and it is meeting the standard specified in official limits.

Drug and excipient compatibility studies were carried out by FT-IR technique to study any possible interaction of excipients with drug in the formulation. FT-IR spectra of drug showed principal peaks at 3548.54 cm-1(OH Stretching), 1441.23 cm-1(NH bending), 1583.74 cm-1(C- O Stretching). Thus, FT-IR studies indicates that there was interaction between the drug and excipients as the principal peak of the drug in the spectra were also observed in the spectra of physical mixture of drug with excipients.

The vesicle size of ethosomes was found to be in a range of 224-396nm.

Percentage entrapment efficiency was found to be in the range of 32.68% to 58.92%. The entrapment efficiency of F6 was found to be maximum whereas F1 was found to be minimum. The data shows that entrapment efficiency of vesicles increases with increase in concentration of ethanol from 25 to 35% w/v and increase in concentration of phospholipid from 1 to 3% w/w.

In-vitro release studies were carried out for all the prepared formulations F1 to F9 & the result were tabulated below.The percentage cumulative drug release of F1-F9 was found to be in the range of 75.39% to 92.06%. F6 showed maximum drug diffusion of 92.06% and was selected as the best formulation. pH of ethosomal gels F6 in G1, G2 and G3 formulations were checked for pH and the values were shown on table 7. The pH of the formulations was found to be in neutral range. Hence the ethosomal gel will not produce any local irritation to the skin. The spreadability of the formulation F6 in G1,G2 & G3 lies in range 26.14±0.17-29.41±0.25. Spreadability of G2 was found to be maximum and were tabulated on table 7.

The viscosity of the formulation F6 in G1, G2 and G3 was determined by Brookfield viscometer, using LV-64 spindle at different rpm and results were given in table 7. Drug content of the prepared formulation F6 in G1, G2 and G3 was found to be in a range of 88.10-95.43%. G2 shown higher drug content of 95.43±0.16% and results were on table7. From Ex-vivo skin permeation study, the permeation of the drug was found to be 85.84% by using rat abdominal skin.

The drug release kinetics showed highest regression value for first order kinetics. The data obtained from Korsmeyer- Peppas, the value of ‘n’ was found to be 1.0216 which indicates that the mechanism of drug release was super case II transport. Stability studies was carried out for the selected best formulation at two different temperature i.e. 4±2°C/40%RH and 25±2°C/60±5% RH for 3 months. Formulation was periodically evaluated for visual appearance, entrapment efficiency, drug content and In-vitro drug release. The results showed that there is no much significant difference in the results.

The vesicle shape of ethosomal formulations were found to be round and was well identified. The turbidity of all formulations was determined by keeping 500 NTU as standard and turbidity of ethosomes is given in table 5.

Formulation Code	Drug (mg)	Soyalecithin (%w/w)	Cholestrol (%w/w)	Ethanol (%w/v)	Propylene glycol (%w/v)
F1	50	1	0.005	25	10
F2	50	2	0.005	30	10
F3	50	3	0.005	35	10
F4	50	1	0.005	25	10
F5	50	2	0.005	30	10
F6	50	3	0.005	35	10
F7	50	1	0.005	25	10
F8	50	2	0.005	30	10
F9	50	3	0.005	35	10

Table 1: Formulation table of Vildagliptin ethosomes

INGREDIENTS	FORMULATIONS			
	G1	G2	G3	PD
Ethosomal suspension (%w/v)	10	10	10	10
Carbopol (%w/v)	1	1.5	2	1.5
Triethanolamine (%w/v)	0.5	0.5	0.5	0.5
Aqueous phase (%w/v)	q.s	q.s	q.s	q.s

Table 2: Formulation table of Vildagliptin ethosomal gel

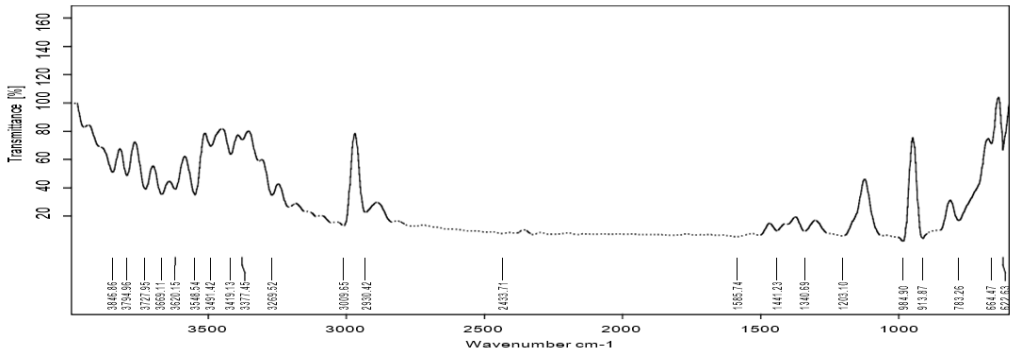

Figure 1: *FT-IR* Spectrum of drug Vildagliptin

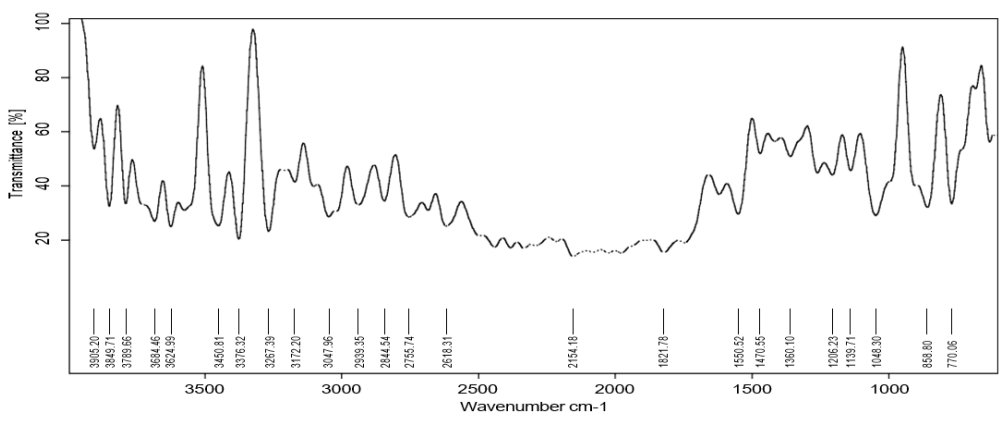

Figure 2: FT-IR Spectrum of Vildagliptin + Soya lecithin+Cholestrol

Formulations	F1	F2	F3	F4	F5	F6	F7	F8	F9
Vesicle size (nm)	323	343	396	245	395	224	316	254	300

Table 3: Vesicle size of Vildagliptin ethosomal formulation

Formulations	F1	F2	F3	F4	F5	F6	F7	F8	F9
%Drug entrapped	32.68	43.31	48.61	40.2	48.32	58.92	41.48	47.99	51.52

Table 4: % Drug entrapment efficiency of ethosomal formulation

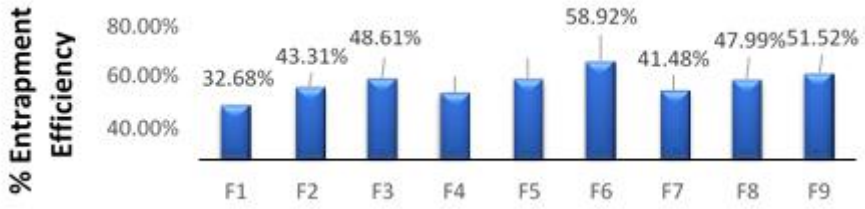

Figure 3: Comparison of entrapment efficiency of F1-F9 formulations

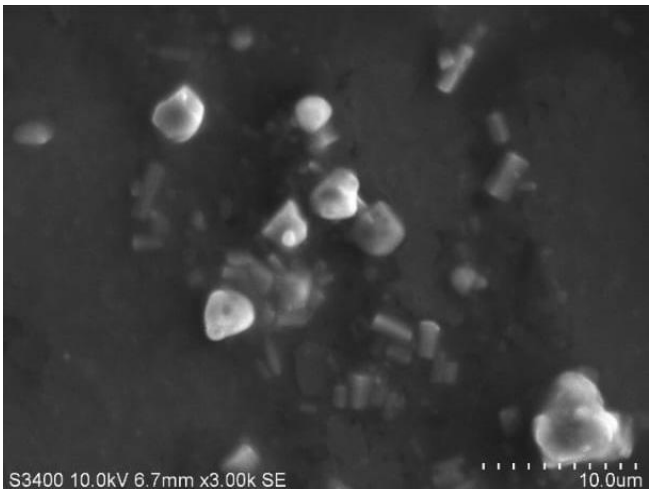

Figure 4: SEM analysis of ethosomal formulation Turbidity Measurement

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Turbidity (NTU)	310	342	373	330	264	253	409	426	430

Table 6: In-vitro diffusion study of formulation F1-F9

Time (hr)	% CDR								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1.	13.43	16.69	15.06	17.91	15.67	14.45	12.72	15.36	16.55
2.	19.14	19.11	18.92	19.62	17.58	19.20	18.78	18.29	18.81
3.	22.76	21.65	23.19	25.97	25.06	22.38	21.93	29.37	21.55
4.	29.89	28.27	27.26	29.22	27.52	25.85	26.92	35.62	25.32

5.	35.43	33.16	38.29	34.35	30.07	35.52	32.14	39.56	36.92
6.	39.07	39.33	47.02	44.39	34.34	43.14	39.94	43.52	43.11
7.	43.03	44.62	55.91	50.09	40.24	52.32	43.69	49.78	52.58
8.	47.92	50.13	64.22	54.91	44.51	57.71	46.86	57.05	57.42
9.	59.99	54.86	74.31	64.65	55.01	64.30	51.43	64.86	62.28
10.	63.48	61.94	78.99	69.95	63.32	81.11	58.3	69.75	68.29
11.	71.12	69.68	85.18	74.36	71.29	88.09	61.76	77.56	75.72
12.	75.39	84.64	90.78	77.82	89.54	92.06	82.2	87.79	88.94

Table 6: In-vitro diffusion study of formulation F1-F9

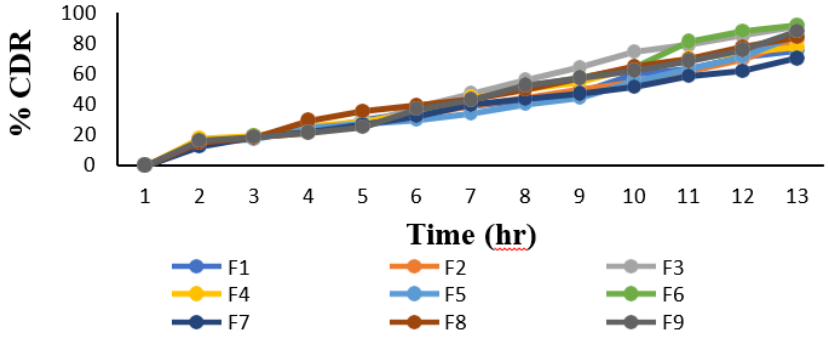

Figure 5: In-vitro diffusion plot of F1 – F9 Vildagliptin ethosomal formulations The In-vitro diffusion studies of Vildagliptin ethosomal gels containing different concentrations of phospholipid, cholestrol, ethanol was carried out for 12hrs.

Formulations	pH	Spreadability (gcm/sec)	%Drug content	Viscosity (cps) 12 rpm
G1	7.0± 0.231	27.76± 0.43	93.96%	11000
G2	7.1± 0.134	29.41± 0.25	95.43%	11300
G3	6.97 ± 0.202	26.14± 0.17	94.10%	11500

Table 7: Evaluation of Ethosomal Gel

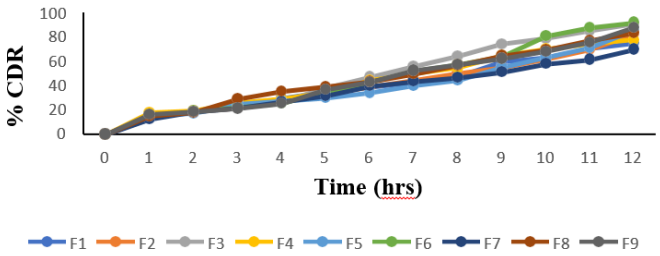

Figure 6: Zero order plot of F1- F9 formulations

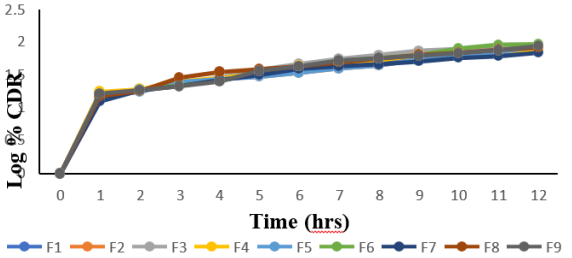

Figure 7: First order plot of F1- F9 formulations

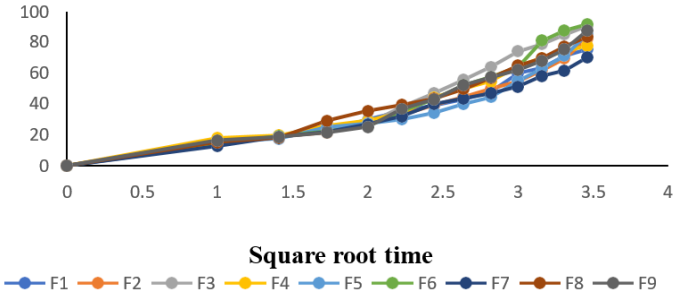

Figure 8: Higuchi plot of F1- F9 formulations

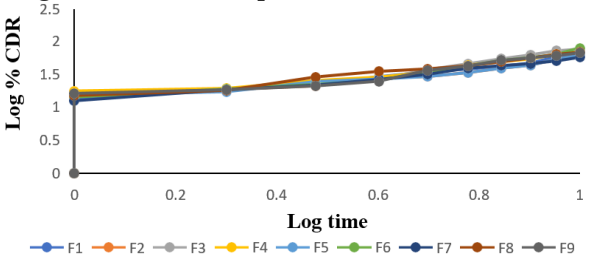

Figure 9: Korsmeyer Peppas plot for F1- F9 formulations

CONCLUSION

In this study, total nine Vildagliptin ethosomal formulations was prepared by using cold method. On the basis of different parameters such as entrapment efficiency of 58.92%, In-vitro diffusion of 92.06%, vesicle size and spreadability F6 was selected as the best formulation and it was further incorporated into gel by using carbopol 934 and carbopol containing 1.5% w/w showed maximum drug release of 93.34%. Stability studies also given satisfactory results as no significant changes were observed. Thus, ethosomes were found to be promising candidate for transdermal delivery of Vildagliptin.

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