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Formulation and Evaluation of Proliposomal Gel Containing Repaglinide Using Mannitol as Water Soluble Carrier

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Abstract

Aim: *The aim of the present investigation was to design a proliposomal gel system of Repaglinide for the treatment of type - 2 diabetes mellitus that is capable of delivering entrapped drug over an extended period of time.*
Method: *Proliposomes of Repaglinide were prepared by thin film hydration technique by varying the composition mannitol, soya lecithin and cholesterol. Proliposome formulations were characterized for compatibility, vesicle size, drug content, entrapment efficiency, surface morphology, zeta potential, DSC investigation, in-vitro drug release and stability studies. The proliposomal gel was prepared for optimized proliposomal formulation F4 by incorporated into 1% carbopol gel. The in-vivo skin irritation study and hypoglycemic activity was carried out for the gel F4-G1.*
Results: *Drug and physical mixture were characterized by FTIR, the result of IR and study showed that no interaction between drug and polymers and other formulation parameters of formulated proliposomes and proliposomal gel are evaluated which showed better results.*
Conclusion: *Proliposomal gel F4-G1 was proved nonirritant and shows better stability, more hypoglycemic effect as compared to oral formulation because it provide reduction in glucose level with controlled manner upto 24 hrs. Hence, proliposomes drug delivery system was better choice for controlled release of drug through topical drug delivery.*
Key words: *Repaglinide, Proliposome, Mannitol, Carbopol, Hypoglycemic, Controlled release*

1. INTRODUCTION:

Drug delivery systems using colloidal particulate carriers such as liposomes and niosomes have distinct advantages over conventional dosage forms. Consequently a number of vesicular drug delivery systems such as liposomes, niosomes, transferosomes, and pharmacosomes and provesicular systems like proliposomes and proniosomes have been developed[1].
Liposomes are the most promising and broadly applicable of all the novel delivery systems. For liposomes to enter the market, they must be stable during the storage period, and remain intact before reaching their targeted tissues to produce action. Various approaches have been used to overcome these problems, some of which include, control of particle size and lamellarity, altering the lipid composition, lyophilisation, electrosteric stabilization etc. One of such approach which helped to overcome the stability issue associated with liposome and led to the development of a new drug delivery system is the Proliposome (PL) discovered by Payne *et al*., in 1986. Proliposomes (PLs) are dry, free-flowing granular products composed of drug(s) and phospholipid(s) which, upon addition of water, disperse to form a multi-lamellar liposomal suspension[2].
Proliposomes an alternative forms to conventional liposomal formulation composed of water soluble porous powder as a carrier, phospholipids and drugs dissolved in organic solvent. Lipid and drug are coated onto a soluble carrier to form free-flowing granular material show controlled release, better stability, ease of handling and increased solubility[3].
Diabetes mellitus is a chronic metabolic disorders characterized by a high blood glucose concentration (hyperglycemia), glycosuria, hyperlipedimia, negative nitrogen balance, and sometimes ketonemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Diabetes is now a days that affects 371 million people worldwide, and 187 million of them do not even know they have the diabetes, according to the International Diabetes Federation (IDF). Researchers estimate that the diabetes dilemma will only increase. By 2030, they expect 552 million people will have the disease[4, 5].

Repaglinide is an oral blood glucose lowering drug of Meglitinide class used in the management of

type 2 diabetes mellitus (NIDDM). Repaglinide acts by closing ATP-dependent potassium channels in the beta cell membrane by binding at characterizable sites. The potassium channel blockade depolarizes the beta cell, which leads to an opening of calcium channels. Their resulting increased calcium influx induces insulin secretion[6].

Repaglinide, a fast and short-acting Meglitinide analog was chosen as the drug candidate since it is indicated for the development of a dosage form with increased gastric retention time. Repaglinide has an extremely short half-life of 1hr. In addition, the oral bioavailability of Repaglinide is low (56%) due to poor absorption in the upper intestinal tract and extensive hepatic first-pass effect after either an IV or oral dose. Moreover it produces hypoglycemia after oral administration. Dosage frequency of Repaglinide is 0.5 to 4 mg in 3 to 4 times in a day. It has melting point of 130-131 ℃ and Mol. wt. 452.58. It belongs to class BCS class-II compound with poor solubility and high permeability. These properties make it suitable for transdermal delivery[7, 8].

Hence, in the present investigation, an attempt is made to formulate Repaglinide loaded proliposomal gel in order to increase bioavailability and reduce side effects by achieving transdermal drug delivery.

2. MATERIALS AND METHOD

Materials

Repaglinide was gifted from Biocon Ltd. Karnataka and Mannitol was purchased from Medreich Ltd. Karnataka, Soya lecithin was purchased from Pharma Sonic Biochem Extractions Ltd. Indore, Cholesterol, Carbopol 934, Alloxan and other solvent like Triethanolamine, Chloroform and Methanol purchased from S d fine chem Ltd. Mumbai.

2.1 PREFORMULATION STUDIES

2.1.1 Melting point determination:

Digital melting point apparatus was used for the determination of melting point of Repaglinide. A few quantity of Repaglinide is taken and placed in a thin walled capillary tube about 8 - 10 cm long and 1 mm inside diameter and closed at one end then is suspended into an oil bath containing silicone oil. The apparatus can be heated slowly, the temperature range over which the sample is observed to melt is taken as the melting point.

2.1.2 Solubility analysis:

Solubility analysis was carried out for Repaglinide samples in various solvents. 10 mg of Repaglinide was dissolved in 10 ml of different solvents i.e. water, phosphate buffer pH 1.2, 6.8, 7.4, chloroform, methanol, and ethanol. Solubility was determined on the basis of physical appearance.

2.1.3 Drug-Excipients compatibility studies by FT-IR

Drug-excipients compatibility studies were carried out using FT-IR. Infrared spectrum of pure drug, mannitol, soya lecithin, cholesterol and the physical mixture of drug:mannitol:soyalecithin:cholesterol in 1:1:1:1 was recorded in between 400 to 4000 cm^{-1} by using liquid sampling technique.

2.2 METHODS

2.2.1 Preparation of Repaglinide proliposome:

Proliposome formulation containing Repaglinide was prepared by using thin film deposition on carrier method using vacuum rotary evaporator**.** Optimization of proliposome formulation was done by preparing varying concentration of water soluble carrier (mannitol), and different ratio of lecithin and cholesterol. 1gm of water soluble carrier (sieved with 100 meshes) was placed in round bottomed flask at 60 - 70 ℃ and 115 rpm under vacuum 30 min for complete drying. Repaglinide 100 mg, lecithin and cholesterol were dissolved in mixture of chloroform and methanol in the ratio of 8:2 (v/v) for various formulations as shown in Table 1. Initially 5 ml aliquot of organic solvent was introduced into round bottomed flask at 37 ℃ and rotated, after complete drying second aliquot 5ml of solution was used. This process was repeated until the solution (10 ml) was used up. The flask containing proliposome formulation was kept in vacuum desiccator overnight and then sieved with 100 meshes.

Table 1: Formulation design for the preparation Repaglinide proliposomes

Formulation Code	Drug (mg)	Mannitol (mg)	Soya lecithin (mg)	Cholesterol (mg)
F1	100	1000	50	50
F2	100	1000	100	50
F3	100	1000	200	50
F4	100	1000	200	75

2.2.2 Preparation of carbopol gel:

1 gm of Carbopol 934 was weighed then dispersed in water with mild stirring and allowed to swell for 24 hrs. To obtained 1% gel and triethanolamine was added to bring the pH neutral.

2.2.3 Preparation of Repaglinide proliposomal gel:

1gm of proliposome formulation was dissolved in 10 ml of methanol and centrifuged (REMI, India) at 6000 rpm for 20 min to remove the unentrapped drug. The supernatant was decanted and sediment was incorporation of the proliposomes into 1% carbopol gel was achieved by slow mechanical mixing using mechanical stirrer (Remi motors) at 25 rpm for 10 min. The optimized F4 formulation was choose for gel preparation that is F4-G1 [9].

3. Evaluation of Repaglinide proliposomes:

3.1 Vesicle size analysis:

A drop of distilled water was added to few proliposome granules on glass slide without cover slip to observe the formation of liposome from proliposome formulation and vesicle size analysis was carried out using an optical microscope with a calibrated eye piece micrometer. About 300 liposomes were measured individually, average was taken and their size distribution range and mean diameter were calculated. The particle size of the vesicle after hydration of proliposomes was also determined by Malvern Zeta potential Analyzer for the optimized formulation.

3.2 Surface morphology by scanning electron microscopy (SEM):

The surface morphology of Repaglinide proliposomes, they were determined by scanning electron microscopy (SEM). Proliposomes was coated with Gold-palladium alloy coating done by sputter coater (Polaron SEM coating system). Then the samples were observed under the scanning electron microscopy (JSMT330A, JEOL) at a beam voltage of 15 kV.

3.3 Zeta potential:

A surface charge of proliposome formulation of the optimized batches was dissolved in distilled water and made a higher serial dilution 1000 X until a clear solution is obtained. Sample was analyzed using Malvern Zeta Analyzer for determination of Zeta potential[10].

3.4 DSC investigation

DSC investigation was carried out for the pure drug and optimized formulation to confirm the incorporation of drug in to the carriers like mannitol for the proliposome formation. Thermal characteristics of the pure drug and for optimized formulation were performed by using an automatic thermal analyzer system (Mettler DSC 823, Germany). The entire samples were run at a scanning rate of 10 ℃ per min from 25 ℃ – 300 ℃ [11].

3.5 Drug content:

100 mg of proliposomes formulation were dissolved in 10 ml methanol by shaking the mixture for 5 min. The clear solution after suitable dilution was measured by U.V spectrophotometer against blank at λmax 226 nm and the drug content was calculated.

3.6 Entrapment efficiency:

The entrapment efficiency of Repaglinide proliposomes was determined after hydration with distilled water. 2 ml of distilled water was added to the proliposome equivalent to 2 mg of drug then the mixture was shaken manually for 2 min. For the separation of unentrapped Repaglinide, the liposomal suspension was subjected to centrifugation on a cooling centrifuge (REMI TR-01) at 25000 rpm for 1 hr. for the separation of unentrapped drug. The clear supernatant (0.1 ml) was taken and diluted to 10 ml with phosphate buffer pH 7.4 and absorbance was recorded at 226 nm using UV-visible spectrophotometer then calculates the percentage drug in the each formulation.

Percentage entrapment (%) = C_t - C_f / C_t × 100

C_t = Total drug content

C_f = Free drug content

3.7 *In Vitro* drug release studies:

The release of drug was determined by using the treated cellophane membrane mounted on the one end of open tube, containing proliposomes (equivalent to 16 mg Repaglinide). The dialysis tube was suspended in 500 ml beaker, containing 250 ml phosphate buffer pH 7.4. The solution was stirred at 100 rpm with the help of magnetic stirrer at 37 ± 0.5 ℃. Perfect sink conditions were maintained during the drug release testing. The samples were withdrawn at suitable time interval (at 1, 2, 4, 6, 8, 12, 16, 20 and 24 hrs). The dissolution medium was replaced with same amount of fresh phosphate buffer pH 7.4 solutions to maintain the volume 250 ml throughout the experiment. The drug content in the withdrawn samples (5 ml) were analyzed by UV spectrophotometer at λmax 226 nm after making the volume up to 10 ml with phosphate buffer pH 7.4 and cumulative % of drug released was calculated and plotted against time (t). The rate and release mechanism of Repaglinide from the prepared proliposomes were analyzed by fitting the release data in to various kinetic models[12].

3.8 Stability studies:

Accelerated stability testing studies was performed for 6 months as per ICH guidelines. The optimized formulation was kept at 40 ± 2 ℃ and 75 ± 5 % RH in stability chamber. Regular tested for % entrapment, vesicle size and drug release were fixed as physical parameters for stability testing[13].

4. Evaluation of Repaglinide gel:

4.1 Physical examination:

The prepared proliposomal gel formulations were inspected macroscopic examination for visual physical appearance of color, consistency texture,

greasiness and clarity was determined by using clarity chamber with black and white background. These all features were done for proliposomal gel formulation**.**

4.2 Measurement of pH:

Triethylamine was used to neutralize the gel and the pH of various gel formulations was determined by using digital pH meter. The glass electrode first calibrated with two standard buffers pH 4.0 and pH 7.0 solution then measurement of pH of each formulation was done in triplicate and average values were calculated.

4.3 Viscosity and Rheological properties:

Brookfield digital viscometer was used to measure the viscosity (in cps) of the prepared gel formulation[14].

4.4 Drug content:

2 gm. proliposomal gel sample was withdrawn from container and dissolved in 100 ml ethanol. After suitable dilution absorbance was measured by U.V spectrophotometer against blank at λmax 226 nm and the drug content was calculated.

4.5 *In Vitro* release studies:

An *in vitro* drug release study was performed using modified Franz diffusion cell. Egg membrane was placed between receptor and donor compartments. Proliposomal gel equivalent to 1 gm 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 ℃ with stirring at 500 rpm throughout the experiment. At fixed time interval, 5 ml of aliquots was withdrawn for every 1, 2, 4, 6, 8, 12, 16, 20 and 24 hrs from receiver compartment through side tube and analyzed by UV spectrophotometer at λmax 226 nm[15].

4.6 *In vivo* studies:

4.6.1 Skin irritation test:

The two young Wister albino rats were taken for skin irritation studies. Hair on the back area (approximately 6 cm^2 area) of each rat was removed by hair removing cream. Developed formulations were applied to the shaved area, and then rats were secured. The animal were observed and evaluated for any sign of erythema or edema for a period of 7 days. The area examined for any signs of skin sensitivity or irritation were secured at the same site at 1^{st}, 3^{rd}, 7^{th} day. All the respective treatments were continued till 7 days and finally application sites were monitored visually and graded according to the visual scoring scale as in Table 2 [16].

Table 2: Standards for skin irritation study

SKIN RESPONSES	SCORE
Erythema and scar Formation	
No Erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight scar formation (injuries in depth)	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1.0 mm)	3
Severe edema (raised more than 1.0 mm and extending beyond	4
Total possible score for irritation	8

4.6.2 Hypoglycemic activity:

Experimental Animal model

Adult healthy Wistar Albino rats of either sex weighing (150-180 gm) were selected for the study. The animals were randomly distributed into various groups and housed individually in polystyrene cages and a specific room at a temperature of 25 ± 2 ℃ and 50 ± 5 % relative humidity, under standard environmental conditions 12 hrs light and 12 hrs dark cycle and animals were acclimatized to laboratory hygiene conditions for l week before the start of experiment. The animals were fed with standard rodent diet and water *ad libitum* throughout the experiment. All procedures described were reviewed and approved by the Institutional of Animal Ethical Committee (IAEC) of Bharathi College of Pharmacy. (Reg.No. BCP/IAEC/CEU/02/ 2015).

Induction of diabetes:

The acclimatized rats were kept fasting for 24 hrs with water *ad libitum* and injected intraperitoneally a dose of 120 mg/kg of Alloxan monohydrate in normal saline. After 1 hr, the rats were provided

feed *ad libitum*. The blood glucose level was checked before Alloxanisation. Then Alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, hence rats were treated with 5 % glucose solutions in bottles kept for the next 24 hrs in their cages to prevent hypoglycaemia. Then each rat blood glucose level was measured by using digital glucometer (ACCU-CHEK Active) after 24 hrs. Rats showing 200 – 250 % increase in fasting blood glucose levels were selected for study[17].

Preparation of animals for studies:

Hairs on the backside (interscapular region) of the rats were removed with a depilatory cream and treatment was provided topically on shaved area. Prior to, day of the experiment, animals were divided into 3groups (n=3) of diabetes rats and 1group (n=3) of normal rats. The rats as treated as following.

Group I - Normal control and was treated with normal 0.9 % saline solution.

Group II - Diabetic control rats received 0.9 % saline solution

Group III - Hyperglycemic rats received oral dose 0.2 mg/kg Repaglinide solution.

Group IV - Hyperglycemic rats received proliposomal gel contains Repaglinide (F4-G1).

The blood was be withdrawn by pricking the rat's tail at appropriate time interval for 24 hrs and blood glucose level was measured immediately by using digital glucometer[18, 19].

4.7 Accelerated stability study:

Accelerated stability testing studies was performed for 6 months as per ICH guideline. The optimized formulations were kept at 40 ± 2 ℃ and 75 ± 5 % RH. Physical appearance, drug content and % drug release were fixed as evaluation parameter for stability study.

Results and Discussion

Repaglinide is a white powder. It is practically insoluble in water, freely soluble in methanol, ethanol, chloroform, and soluble in phosphate buffer pH 1.2, 6.8, 7.4. Melting point was determined by digital melting point apparatus and it was found to be 131 ℃, which complied with IP standards, thus indicating the purity of drug. FTIR spectra of pure Repaglinide showed sharp characteristic peaks at 3309.96, 2931.90, 2800.73, 1774.57, 1566.25, 1381.08, 1296.21, and 1087.89 cm^{-1}. FTIR characteristic peaks of pure drug are also observed in the spectra of physical mixture indicating no modification for interaction between the drug and excipients. This proves that there is no potential incompatibility with the drug and the excipients used in the proliposome formulation. Comparative study of FTIR graphs are showed in Fig. 1 and 2

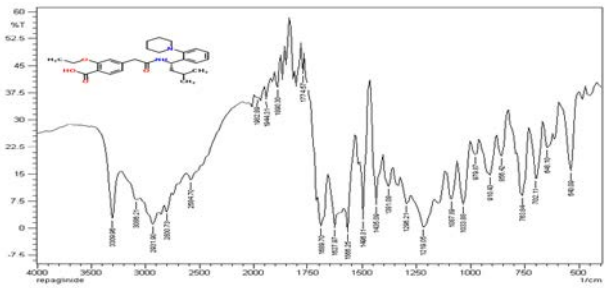

Fig. 1: FT-IR Spectroscopy of Repaglinide

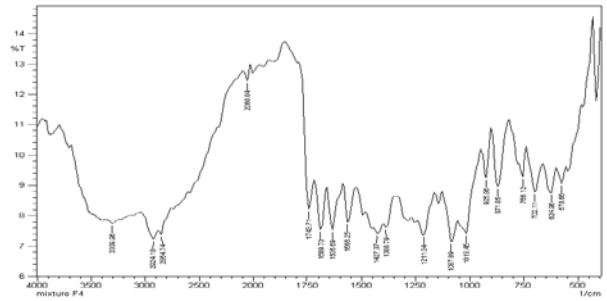

Fig. 2: FT-IR Spectroscopy of Repaglinide+Mannitol+Soya lecithin+Cholesterol

Proliposomes were prepared using film deposition on carrier method using vacuum rotary evaporator**.** The result showed in Table 3. We observed that, increase in the concentration of soya lecithin and cholesterol vesicle size, % drug content and % entrapment efficiency was found to be increased.

Table 3: Vesicle size, % Drug content and % Entrapment efficiency of proliposomes formulations

Formulation code	Average vesicle size in µm	% Drug content	% Entrapment efficiency
F1	2.33	95.63	86.21
F2	3.35	96.47	87.32
F3	4.42	97.23	88.52
F4	5.47	98.56	89.72

The vesicle size of F4 formulation from particle size analyzer was found to be 762.3 nm as shown in Fig. 3

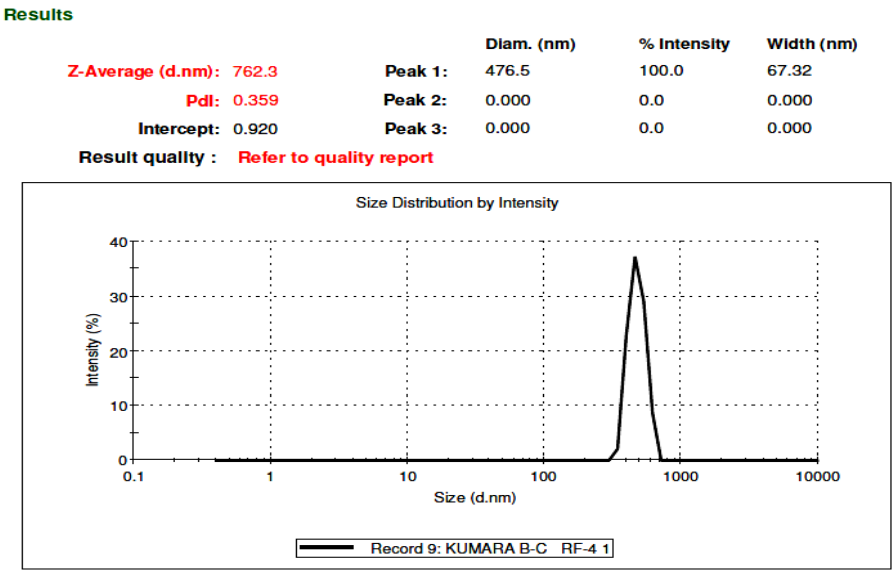

Fig. 3: Particle size data for proliposomes formulation F4

The surface morphology was studied by Scanning electron microscopy (SEM), the SEM photographs of optimized proliposomes formulation F4 as shown in Fig. 4 The crystalline structure of mannitol is modified and porous structure in the images confirmed the formation proliposomes that is confirmed the incorporation of lipids and drug.

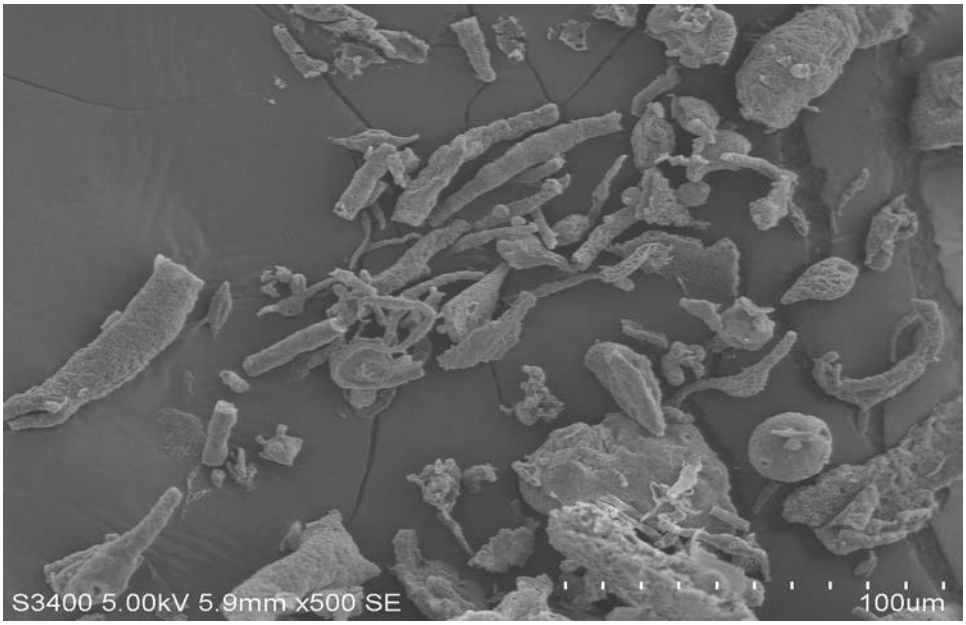

Fig. 4: Scanning electron micrograph of proliposomes formulation F4

Zeta potential of optimized formulation F4 was found to be -36.3 mV, which indicates that the formulation is good to be stable as shown in Fig. 5

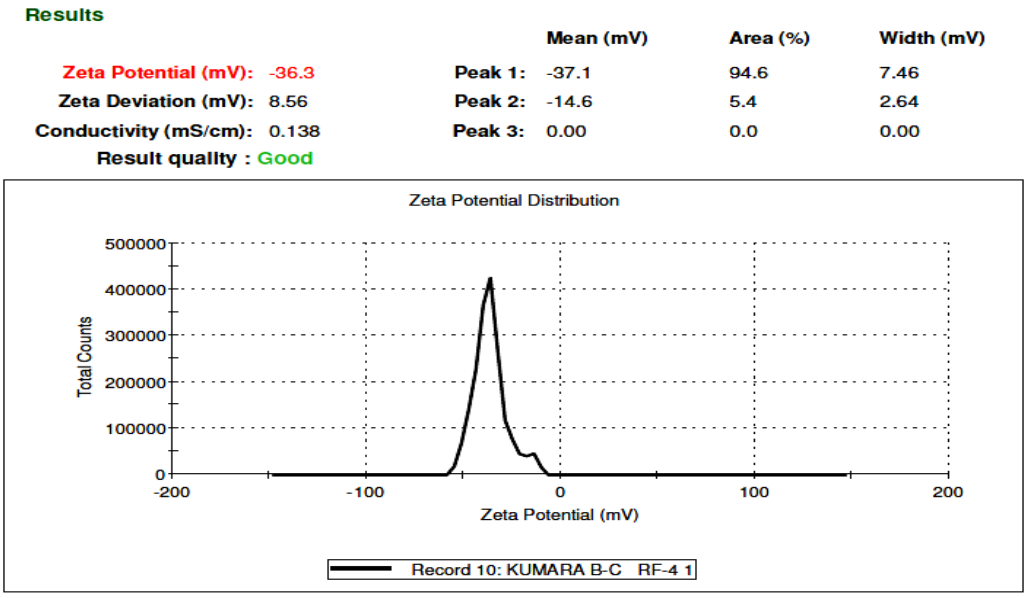

Fig. 5: Zeta potential of optimized proliposomes formulation F4

The DSC investigation of the thermo gram obtained for the pure drug and optimized formulations are shown in (Fig. 6 and 7). Absence of endothermic peak of pure drug in the optimized formulation F4 confirmed the entrapment of drug in the proliposome carriers.

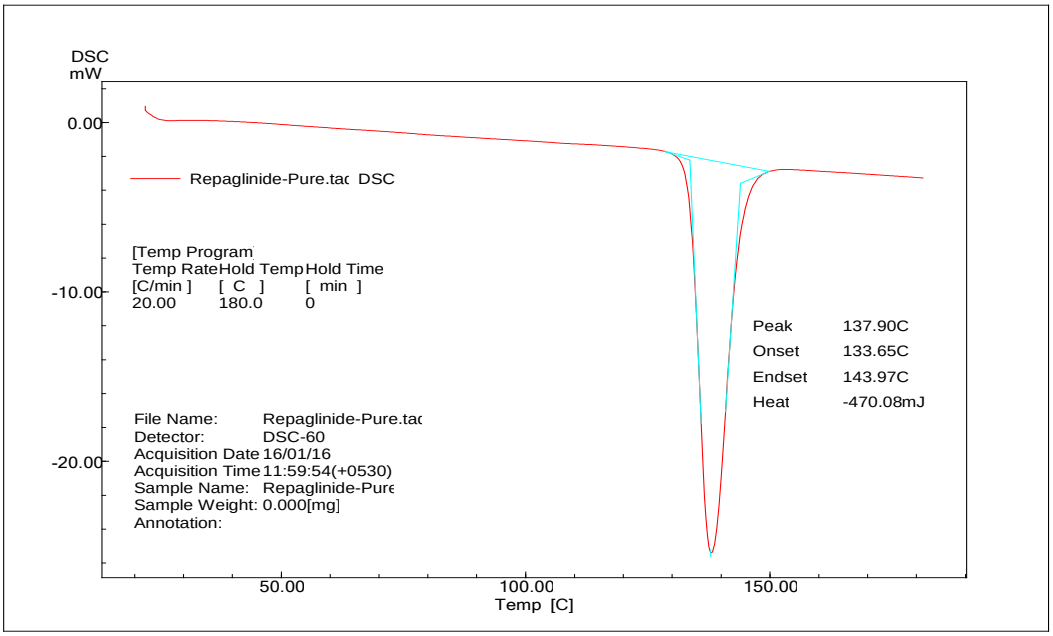

Fig. 6: DSC thermo graph of pure drug Repaglinide

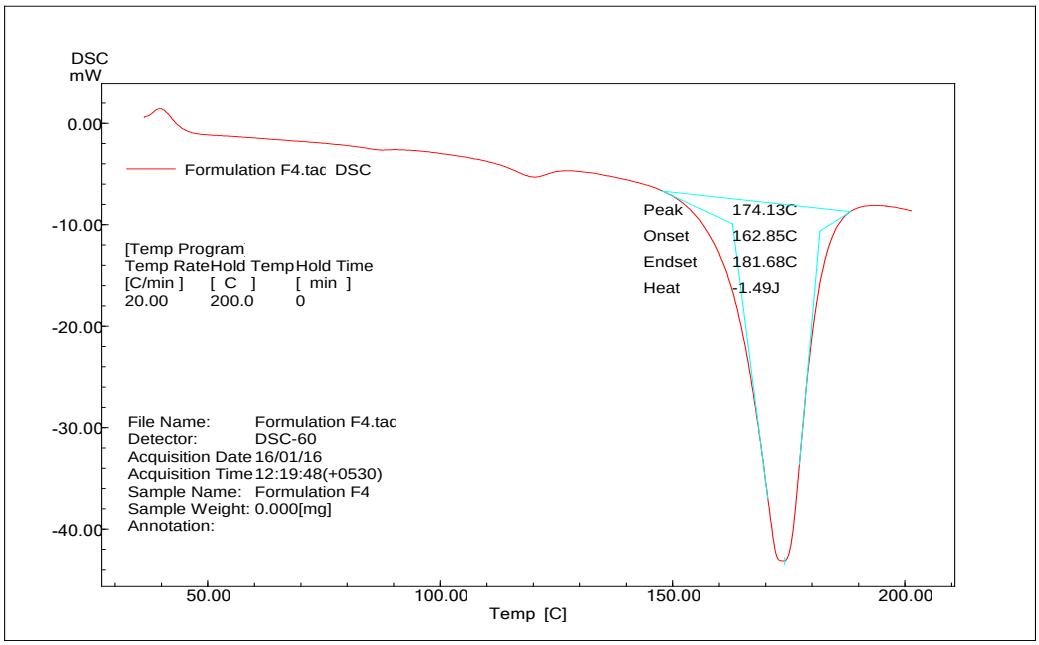

Fig. 7: DSC thermo graph of optimized formulation of F4

The release of drug from proliposomes formulation was varied according to concentration of soya lecithin and cholesterol. The progressive decrease in the amount of drug diffused through a dialysis membrane from formulations F1 to F4 attributed to gradual increase in soya lecithin and cholesterol content. It has been concluded that, if we increase the concentration of soya lecithin and cholesterol, the diffusion of drug also decreases. The amount of drug diffused from formulation F4 was showed 58.25 % which was lower among the formulations F1 to F4 and showed in Fig. 8 and which follows Higuchi model. The 'n' values for all the formulation were found to be more than 0.5. This indicates that the release approximates non-Fickian diffusion mechanism.

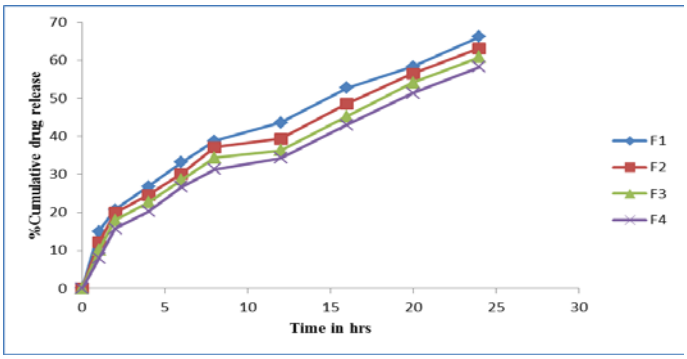

Fig. 8: % Cumulative drug release of proliposomes formulation from F1-F4

Accelerated stability studies were carried out for the most satisfactory formulation F4 at 45 ± 2 ℃ and 75 ± 5 % RH for 6 months. At the end of 1, 3, 6 months intervals, samples were evaluated for different parameters includes vesicle size, entrapment efficiency and % CDR and results indicates that stability studies of selected formulation F4 showed negligible changes in evaluated parameters that revealed that the formulations are stable on storage.

The optimized formulation F4 was incorporated in 1% carbopol 934 gel was prepared. The prepared proliposomal gel formulation was evaluated for the following parameters.

Physical appearance

The Physical appearance of the Repaglinide proliposomal gel formulations was checked and showed translucent, yellowish glossy, smooth and non-greasy on application.

pH measurement

The prepared Repaglinide proliposomal gel was checked for their pH and the formulation was

found to be 6.02. Therefore there is no need for adjusting pH of the formulation.

Viscosity

The viscosity of gels formulation was determined and formulation F4-G1 showed 11250 cps.

Drug content (%)

The prepared Repaglinide proliposomal gel was subjected to drug content uniformity and it was found to 98.55 % which indicated the drug uniformly dispersed throughout the formulation.

***In vitro* drug release**

The result of *In vitro* release of Repaglinide from the gel formulation clearly shows that the gels have ability to retain the drug for prolonged periods. The % CDR of proliposomal gel formulation F4-G1 was found to be 56.25 % as shown in Fig. 9 and which follows Higuchi model. The 'n' values for all the formulation were found to be more than 0.5. This indicates that the release approximates non-Fickian diffusion mechanism and this formulation was selected for next *in vivo* and stability studies.

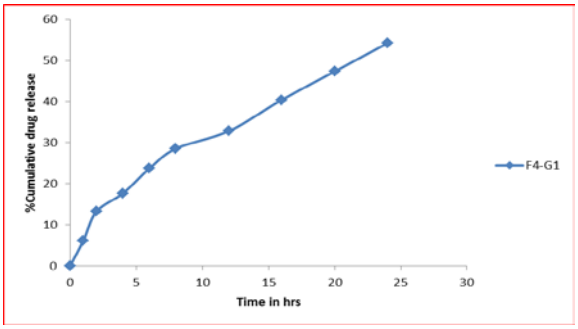

Fig. 9: % CDR of proliposomal gel formulation F4-G1

Skin irritation studies

The skin irritation study of proliposomal gel formulations F4-G1 was performed and tabulated in Table 4. The Average primary irritation index of formulations F4-G1 was found to be 0.16, and it shows that the proliposomal gel formulation did not show any irritation and erythema after 7 days.

Table 4: Reading after Skin irritation study of proliposomal gel formulation F4-G1

Skin responses	Days	F4-G1 Formulation	
		Score	
		Rat 1	Rat 1
Erythema and Scar formation	1	0	0
	3	0	1
	7	1	0
Edema formation	1	0	0
	3	0	0
	7	0	0
Primary irritation index (PPI)		0.16	0.16

Hypoglycemic activity

The results of reduction in blood glucose level of proliposomal gel in comparison with Repaglinide oral, normal and diabetic control rats are shown in Table 5. The blood glucose level reduction in group III at 10 hrs was high with oral administration and observed severe hypoglycemia in the initial hours after administration. Where as, for the proliposomal gel the blood glucose level was reduced in a controlled manner and observed blood glucose level reduction in group IV was showed 154 ± 2.08 at 24 hrs for the optimized formulation F4-G1 was more effective as compared to conventional formulation because it provide reduction in glucose level with controlled manner up to 24 hrs and both normal control group and diabetic control didn't show any reduction in blood glucose level (no hypoglycemic effect).

Table 5: Hypoglycemic activity of Repaglinide proliposomal gel formulation F4-G1

Group-I = Normal control; **Group-II** = Diabetic control; **Group-III** = Oral; **Group-IV** = F4-G1 (Topical)

Time (hrs)	Reduction in blood glucose level in mg/dl (mean ± SD, n=3)			
	Group-I	Group-II	Group-III	Group-IV
0	96 ± 1.00	350 ± 1.52	348 ± 1.52	345 ± 1.52
2	95 ± 1.00	355 ± 1.00	200 ± 1.52	303 ± 1.52
4	95 ± 0.57	358 ± 1.15	150 ± 2.00	275 ± 2.00
8	93 ± 0.57	362 ± 0.57	120 ± 1.52	200 ± 1.52
10	96 ± 0.57	368 ± 0.57	95 ± 1.52	165 ± 1.52
12	95 ± 0.57	367 ± 0.57	180 ± 2.00	157 ± 1.52
24	94 ± 1.00	370 ± 1.00	245 ± 2.08	154 ± 2.08

Stability studies of proliposomal gel

Accelerated stability studies of proliposomal gel formulation F4-G1 shows negligible changes in pH, drug content and % CDR revealed that the formulations are stable on storage.

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