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FORMULATION OF PROLIOPSOMAL GEL CONTAINING REPAGLINIDE FOR EFFECCTIVE TRANSDERMAL DRUG DELIVERY

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ABSTRACT

Objectives: The specific objective of the study is to formulate and evaluate the proliposomal gel of Repaglinide for effective transdermal drug delivery. **Method:** Proliposomes of Repaglinide were prepared by thin film hydration technique by using vacuum rotary evaporator and varying the composition sorbitol, soya lecithin and cholesterol. Proliposome formulations were characterized for compatibility, vesicle size, drug content, entrapment efficiency, surface morphology, zeta potential, *in-vitro* drug release. The proliposomal gel was prepared for optimized proliposomal formulation RF3 and RF6 by incorporated into 1%W/V Carbopol gel. The *in-vivo* skin irritation study and hypoglycemic activity was carried out for the gel PLG1 and PLG2. **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:** The drug-excipients compatibility by FT-IR confirmed no significant interaction between drug and selected excipients. Proliposomal gel PLG1 and PLG2 were proved nonirritant and more hypoglycemic effect as compared to oral formulation because it provide reduction in glucose level with controlled manner upto 24 hr. Hence, proliposomes drug delivery system was better choice for controlled release of drug through topical drug delivery.

KEYWORDS: Repaglinide, Proliposome, Mannitol, Sorbitol, Soya lecithin, Hypoglycemic, Controlled release.

INTRODUCTION

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion, insulin action or both. Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood. Hence blood glucose level increase, which is termed as hyperglycemia. If the glucose level in the blood remains high over a long period of time this can result in long term damage to organ such as the kidneys, liver, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death. 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.[1,2]

Repaglinide is a novel oral blood glucose lowering agent from the class of Meglitinide. It stimulates release of insulin from the pancreatic cell by closure of KATP channels and is rapidly absorbed and eliminated from the body. Repaglinide is developed in attempts to overcome the adverse effects associated with existing antidiabetic compounds. These include hypoglycemia, secondary failure and cardiovascular side effects.[3]

Repaglinide has an extremely short half-life of 1h. 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.[4]

A number of novel drug delivery systems have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structures is one such system, which can be expected to prolong the duration of the drug in systemic circulation, and reduce the toxicity by selective uptaking. 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.[5]

Topical / transdermal drug delivery is an attractive route for local and systemic treatment. Liposomes are the leading in transdermal drug delivery systems for the systemic (intravenous) administration of drugs. Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation.[6]

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.[7]

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.[8]

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

MATERIALS AND METHODS

Materials

Repaglinide was gifted from Biocon Ltd. Karnataka, Mannitol and Sorbitol were 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.

METHODS

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 (sorbitol and mannitol), and different ratio of lecithin and cholesterol. 1 g of water soluble carrier (sieved with sieve no. 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 sieve no. 100 meshes.

Table 1: Formulation design for the preparation Repaglinide proliposomes

Formulation Code	Drug (mg)	Mannitol (mg)	Sorbitol (mg)	Soya lecithin (mg)	Cholesterol (mg)
RF1	100	1000	-	50	50
RF2	100	1000	-	100	50
RF3	100	1000	-	200	50
RF4	100	-	1000	50	50
RF5	100	-	1000	100	50
RF6	100	-	1000	200	50

Preparation of carbopol gel

1 g of Carbopol 934 was weighed then dispersed into water with mild stirring and allowed to swell for 24 h. Then to obtained 1% gel and triethanolamine was added to bring the pH neutral.

Preparation of Repaglinide proliposomal gel

1 g 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 incorporated into 1 % carbopol gel. It was achieved by slow mechanical mixing using mechanical stirrer (Remi motors) at 25 rpm for 10 min. The optimized RF2 and RF5 formulation was choose for gel preparation and coded as PLG1 and PLG2 and used for further studies.[9]

Evaluation of repaglinide proliposomes

The prepared proliposomes were characterized for various parameter like compatibility study, vesicle size, drug content, entrapment efficiency, surface morphology, zeta potential, , *in-vitro* drug release. The *in-vivo* skin irritation study and hypoglycemic activity was carried out for the optimized proliposomal gel formulation.[10]

***In Vitro* drug release studies**

The *in vitro* drug release study was performed using diffusion cell. Synthetic membrane (cellophane) was used as diffusion membrane. The synthetic membrane (cellophane) was soaked for 12 hr in phosphate buffer (pH 6) before subjecting to diffusion study. 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 hr). 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.[11]

Skin irritation test

The Wister albino rats of either sex weighing 150–200 g were taken for skin irritation studies and the intact skin was used for this study. 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 back area and then rats were secured. Gel base was applied to the back area and used as control. The animal were observed and evaluated for any sign of erythema or edema for a period of 7 days.[12]

Hypoglycemic activity

Experimental Animal model

Adult healthy Wister Albino rats of either sex weighing (150-180 g) 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 hr light and 12 hr dark cycle and

animals were acclimatized to laboratory hygiene conditions for l hr w 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 hr 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 hr in their cages to prevent hypoglycaemia. Then each rat blood glucose level was measured by using digital glucometer (ACCU-CHEK Active) after 24 hr. Rats showing 200 – 250 % increase in fasting blood glucose levels were selected for study.[13]

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 hair removed area. The animals were divided into 4 groups (n=5) of diabetes rats and 1 group (n=5) of normal rats. The rats as treated as following.

Group I - Diabetic control rats received 0.9 % saline solution.
Group II - Hyperglycemic rats received oral dose 0.2 mg/kg Repaglinide solution.
Group III - Hyperglycemic rats received proliposomal gel contains Repaglinide (PLG1).
Group IV - Hyperglycemic rats received proliposomal gel contains Repaglinide (PLG2).

The blood was be withdrawn by pricking the rat's tail at appropriate time interval for 24 hr and blood glucose level was measured immediately by using digital glucometer.

RESULTS

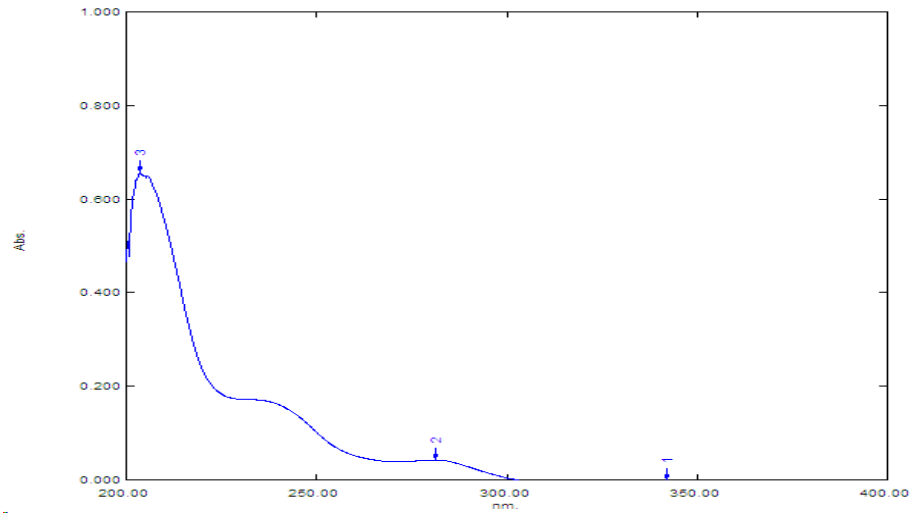

Fig. 1: λmax of the Repaglinide

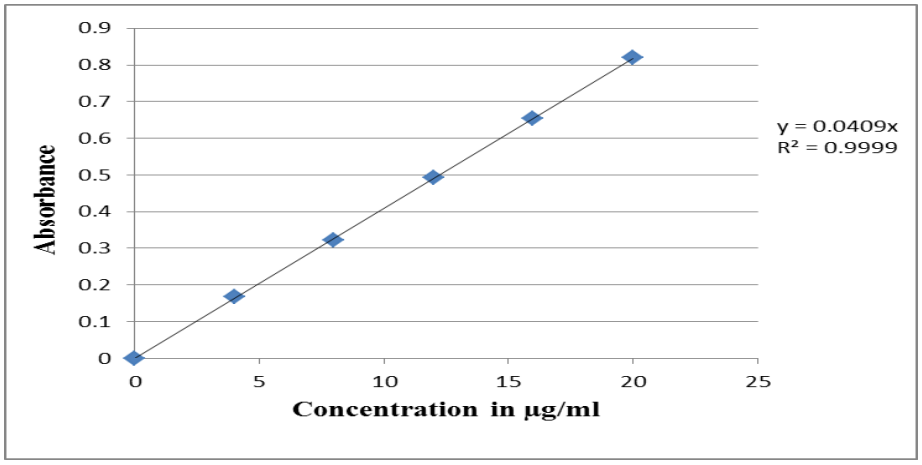

Fig: 2 Standard calibration curve of Repaglinide

Compatibility studies by FT-IR Techniques

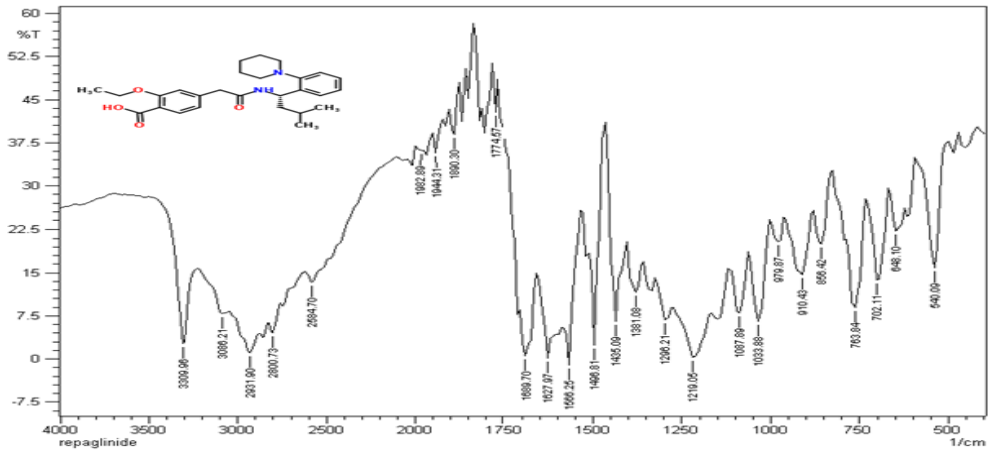

Fig. 3: FT-IR Spectroscopy of Repaglinide

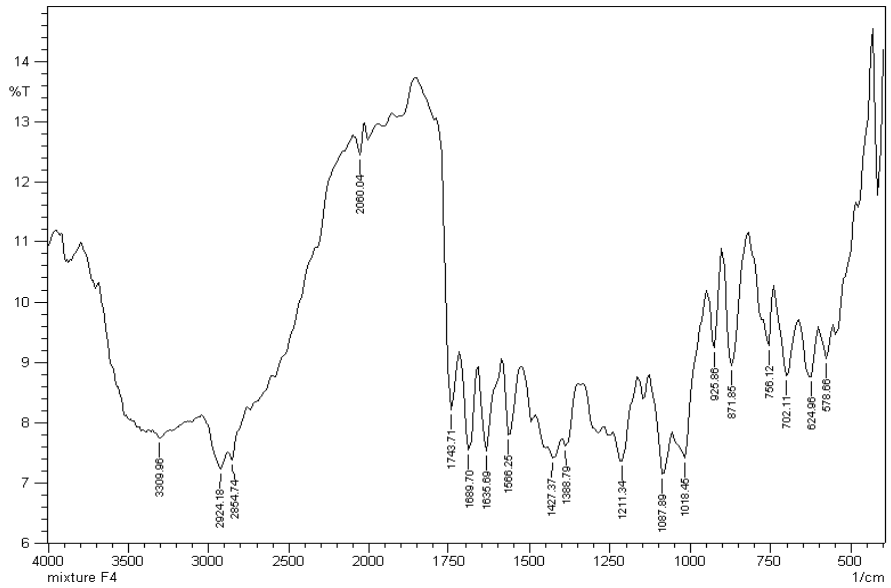

Fig. 4: FT-IR Spectroscopy of Repaglinide+Mannitol+Soya Lecithin+Cholesterol

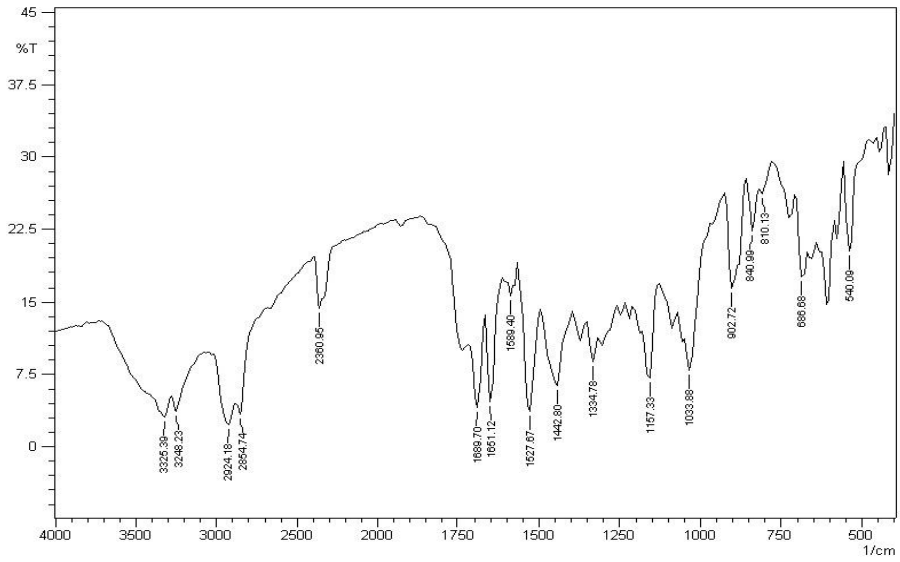

Fig. 5: FT-IR Spectroscopy of Repaglinide+Mannitol+Soya Lecithin+Cholesterol

Table 2: Vesicle size, % Drug content and % Entrapment efficiency of proliposomes formulations

Formulation code	Maximum number of vesicle in size range (µm)	% Drug content	% Entrapment efficiency
RF1	2 - 3	90.63	86.21
RF2	3 - 4	97.47	87.32
RF3	4 - 5	98.23	88.52
RF4	2 -3	95.33	84.82
RF5	3 - 4	98.76	87.77
RF6	4 - 5	99.72	88.78

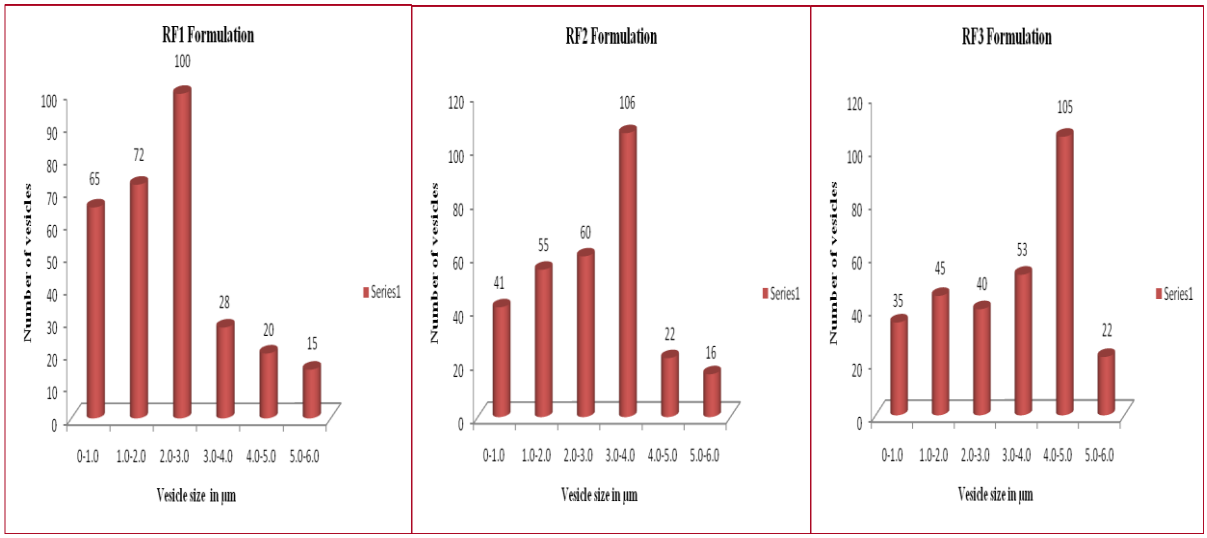

Fig. 6: Particle size data for proliposomes formulation RF1-RF3

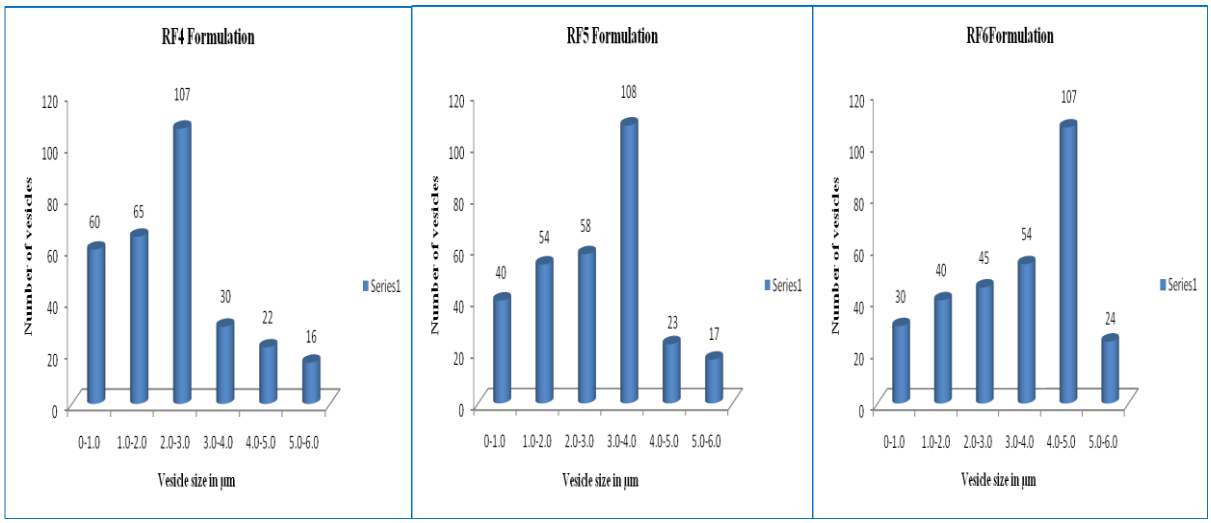

Fig. 7: Particle size data for proliposomes formulation RF4 – RF6

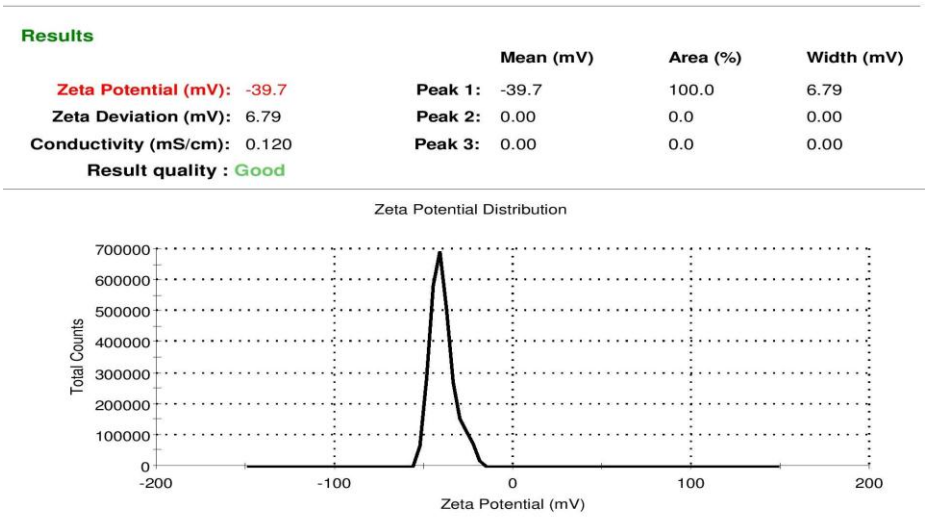

Fig. 8: Zeta potential of optimized proliposomes formulation RF3

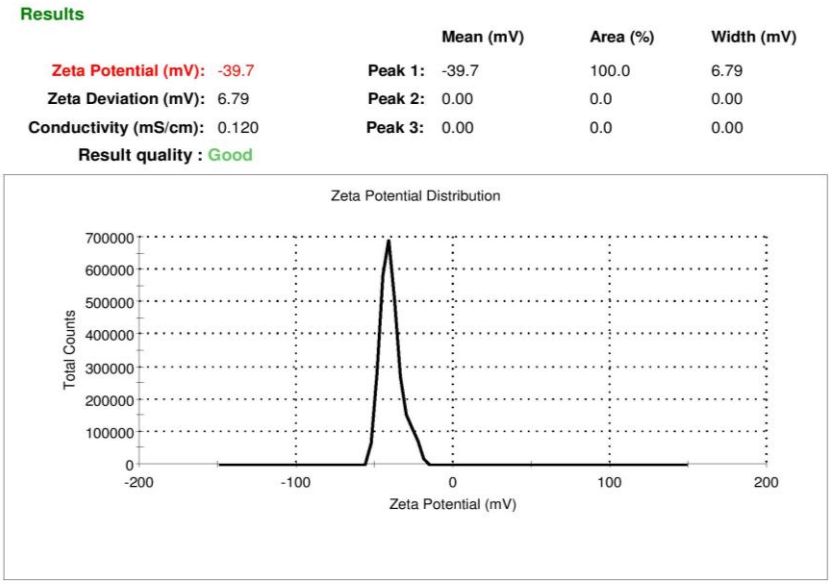

Fig. 9: Zeta potential of optimized proliposomes formulation RF6

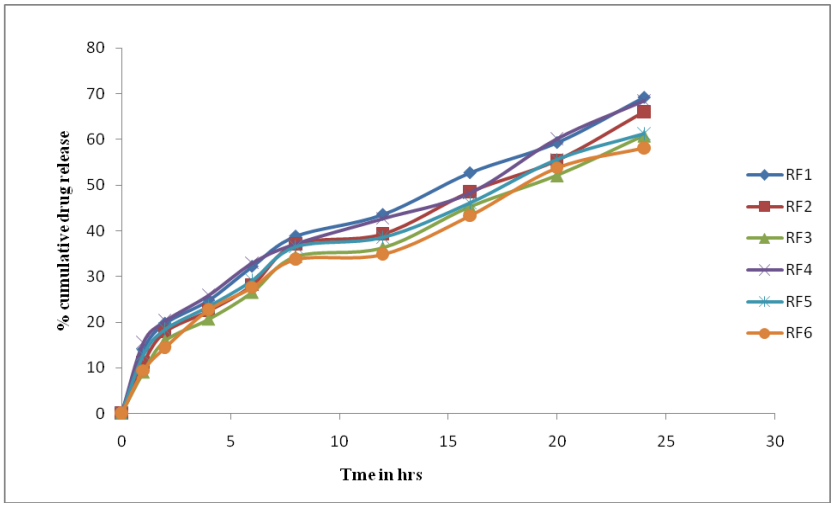

Fig. 10: Cumulative % drug release of proliposomes formulation from RF1-RF6

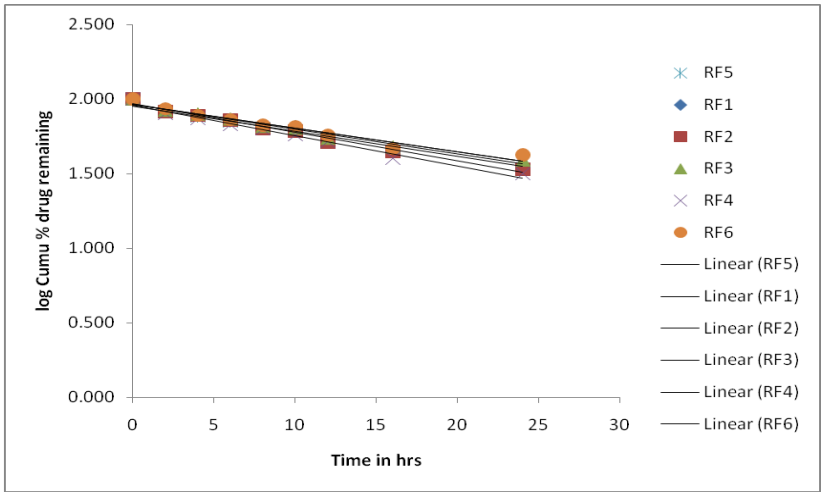

Fig. 11: First order release kinetic profile of formulation from RF1-RF6

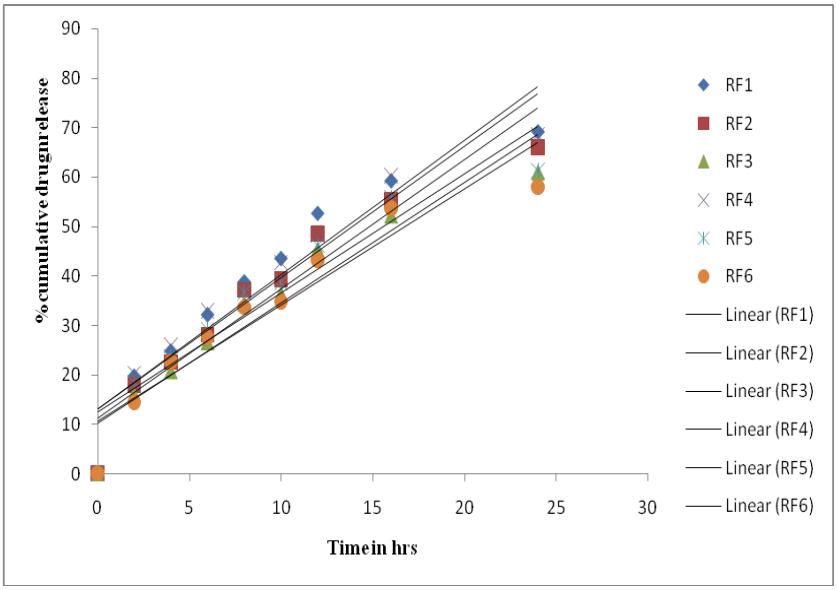

Fig. 12: Zero order release kinetic profile of formulation from RF1-RF6

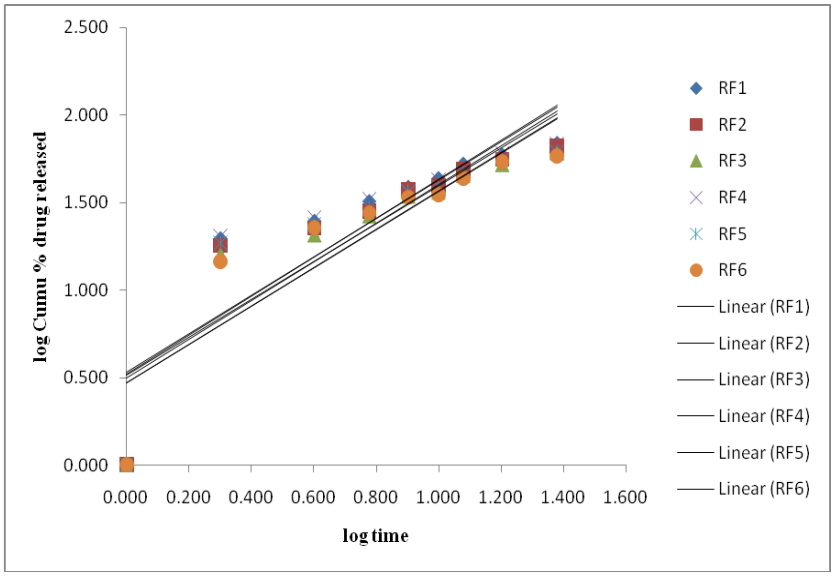

Fig. 13: Peppas release kinetic profile of proliposome formulation from RF1-RF6

Table 3: Release kinetic model fit data

Formulation code	Zero order	First order	Higuchi plot	Peppas plot	
				r^2	'n'
RF1	0.905	0.979	0.989	0.769	1.113
RF2	0.920	0.982	0.986	0.784	1.106
RF3	0.917	0.974	0.985	0.796	1.095
RF4	0.909	0.978	0.991	0.757	1.097
RF5	0.891	0.957	0.987	0.765	1.082
RF6	0.898	0.951	0.981	0.797	1.093

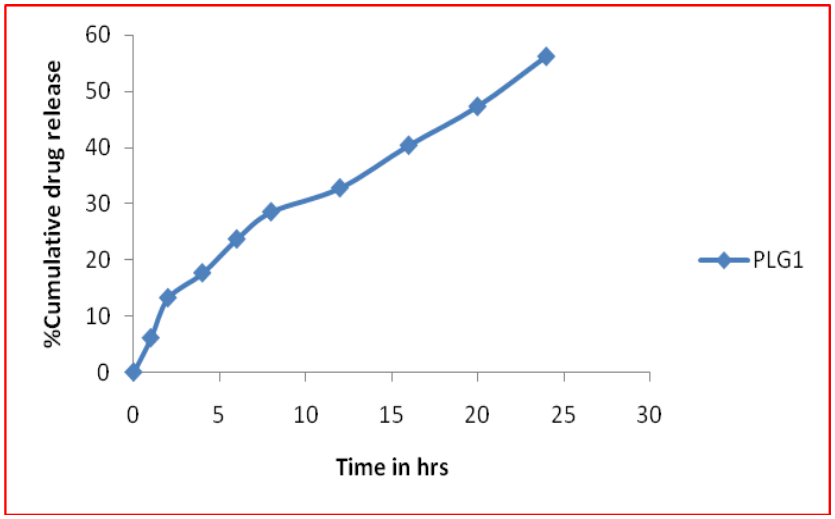

Fig. 14: % CDR of proliposomal gel formulation PLG1

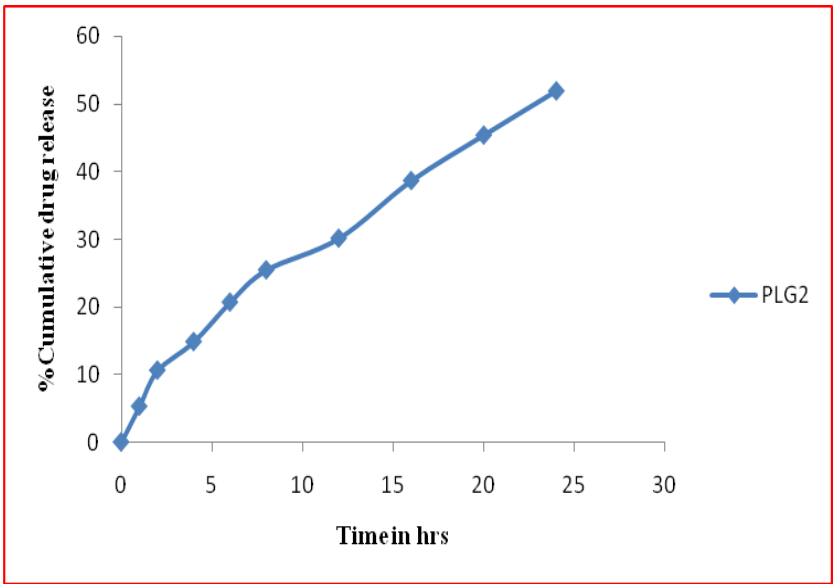

Fig. 15: % CDR of proliposomal gel formulation PLG2

Table 4: Hypoglycemic activity of Repaglinide proliposomal gel formulation PLG1

Group-I Diabetic control; **Group-II =** Oral route; **Group-III** = PLG1 (Topical), **Group IV**=PLG2(Topical)

Time (h)	Reduction in blood glucose level in mg/dl (mean ± SD, n=5)			
	Group-I	Group-II	Group-III	Group IV
0	258±0.54	261±0.70	261±1.14	265±0.54
2	261±0.84	161±0.83	201±0.83	215±0.83
4	264±0.54	141±0.83	171±1.09	180±0.70
8	270±0.54	115±0.83	143±0.83	156±0.83
10	268±0.83	172±0.89	132±0.89	148±0.54
12	271±0.83	200±0.83	121±0.70	140±0.80
24	258±0.54	231±0.83	110±0.70	126±0.83

DISCUSSION

The λmax of the Repaglinide in phosphate buffer pH 7.4 was found to be 226nm and the curve was shown in Fig.1. Standard calibration curve of Repaglinide obeys the Beer's law in concentration range of 4-20 µg/ml in phosphate buffer pH 7.4 with regression of coefficient of 0.9999 and slope of 0.0409 as shown in Fig.2.

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. 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. 3-5.

Different formulations of Repaglinide proliposomes were prepared by thin film deposition on carrier method using Mannitol, Sorbitol, Soya lecithin, Cholesterol, Methanol and Chloroform. Mannitol and Sorbitol was used as a water soluble carrier, Soya lecithin was used as vesicle forming component, Cholesterol improves the stability of bilayer membrane of vesicles and methanol and Chloroform was used as skin penetration enhancer and for providing softness to the vesicles. The prepared formulatiom were evaluated for different parameters.

In Mannitol containing formulation RF1,RF2, RF3 the maximum number of vesicle were observed in the size range of 2 – 3 µm, 3 – 4 µm, 4 – 5 µm respectivel. The results are shown in Fig.6 we observed that ,increase in the concentration of soya lecithin in the formulation RF1-RF3 the vesicle size also increased.In sorbitol containing formulation RF4, RF5, RF6, the maximum number of vesicle were observed in the size range of 2 – 3 µm, 3 – 4 µm, 4 – 5 µm The results are shown in Fig.7, we observed that ,increase in the concentration of soya lecithin in the formulation F5-F7 the vesicle size also increased from 2.65-4.75 µm . In both carriers the vesicle formation behaves in the same manner and observed not much difference in the vesicle size distribution.

Drug content for the prepared formulations was observed with high drug loading, this is more than 90% showing maximum drug content in both Mannitol and Sorbitol containing formulation. The % drug content of formulation RF3 and RF6 showed maximum drug content up to 98.23% and 99.72 %, respectively With maximum entrapment efficiency of

88.52 % and 88.78 % respectivlely as shown in table 2. The % entrapment efficiency was found to increase with increasing the soya lecithin concentration in all the formulation.

Zeta potential of optimized formulation RF2 and RF5 Repaglinide proliposomes are shown in Fig.8 and 9. It was found to be -36.3 mV and -39.7 mV, respectively which indicates that they are sufficient to be stable.

The release of Repaglinide from mannitol and sorbitol as carrier containing proliposomes was varied according to concentration of soya lecithin and cholesterol as shown in Fig 10. The progressive decrease in the amount of drug diffused through cellophane membrane from formulations RF1 – RF3 and RF4- RF5 attributed to gradual increase in soya lecithin ratio. It has been concluded that, if we increase the concentration of soya lecithin the diffusion of drug also decreases. The amount of drug diffused from formulation RF3 was showed 60.84 % which was lower among the formulations RF1 to RF3 and RF6 was showed 58.12% which was lower among the formulation RF4-RF6. The application of different drug release model kinetics is given in Table. 18-25 and release profile represented graphically in Fig. 40-46 it was found that all the formulation 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. Further

The result of *In vitro* release of Repaglinide from the gel formulation PLG1 and PLG2 is given in Fig 14 and 15. However, the results clearly show that the gels have ability to retain the drug for prolonged periods. The %CDR of proliposomal gel formulation PLG1 and PLG2 was found to be 54.25 % and 51.90 %, respectively and follows Higuchi model.

The skin irritation study of proliposomal gel formulations PLG1 and PLG2 was performed and the Average primary irritation index of formulations PLG1 and PLG2 was found to be 0.16 and it shows that the proliposomal gel formulation did not show any irritation and erythema after 7 days.

The results of reduction in blood glucose level of proliposomal gel in comparison with Repaglinide oral, diabetic control rats were shown in Table 4. The blood glucose level reduction in group II at 10 hr was high with oral administration and observed severe hypoglycemia in the initial hours after administration. Whereas, for the proliposomal gel PLG1 (groupIII) and PLG2 (groupIV), the blood glucose level was reduced in a controlled

manner and observed blood glucose level reduction in group III and IV was shown 110±0.70 and 126±0.83 respectively 24 hr. Whereas diabetic control didn't show any reduction in blood glucose level (no hypoglycemic effect). The results confirmed that liposomes are formed by hydration after topical application of proliposomal gel and controlled the drug release over a period of time

CONCLUSION

Topical / transdermal drug delivery is an attractive route for local and systemic treatment. Liposomes are the leading in transdermal drug delivery systems for the systemic (intravenous) administration of drugs. Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. From this study we concluded that proliposomal gel can be potentially used for transdermal application of antidiabetic drugs for the controlled release with improved patient compliance.

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REFERENCES

1. Shankar Murthy K, Kiran BR. Medicinal plants used as an antidiabetic drug in pharmaceutical industry and their conservation: An overview. Int Res J Pharm, 2013; 3(10): 65-71.
2. Wadkar KA, Magdum CS, Patil SS, Naikwade NS. Anti-diabetic potential and Indian medicinal plants. J Herb Med Toxicol., 2008; 2(1): 45-50.
3. Rathore A R, Khambete H and Jain S. Preparation and characterization of Repaglinide loaded ethosomal gel for the treatment of NIDDM. Int j pharm biol sci arch, 2013; 4(2): 385-90.
4. Maulik P Talsania, Shantha Kumar GS, Divakar Goli, Roopa Karkil, Venkatesh. Formulation and optimization of nanostructured lipid matrices of Repaglinide using factorial design. World J Pharm Sci., 2013; 2(6): 5521-27.
5. Deepthi Annakula, Madhukar Rao Errabelli, Raju Jukanti, Suresh Bandari, Prabhakar Reddy, Veera Reddy. Provesicular drug delivery system. Arch Appl Sci Res., 2010; 2(4): 135-46.

6. Nikhil Argan, Harikumar SL, Nirmala. Topical liposomal gel: a novel drug delivery system. IJRPC, 2012; 2(2): 383-91.
7. Bouwstra JA, Honeywell-Nguyen PL. Vesicles as a tool for transdermal and dermal delivery. Drug Discov Today Technol, 2005; 2(1): 67-74.
8. Gitanjali Parmar, Rajni Bala, Nimrata Seth, Angshu Banerjee. Proliposome: Novel drug delivery system. World J Pharm Res., 2015; 4(7): 679-92.
9. Shruthi MV, S.Parthiban, Senthilkumar GP, Tamizmani T. Formulation and evaluation of proliposomal gel containing Metformin hydrochloride using mannitol as a water soluble carrier. Int J Pharm Develop Technol, 2015; 5(1): 27-34.
10. Bo-Young Hwang, Byung-Hwa Jung, Suk-Jae Chung, Min-Hwa Lee, Chang-Koo Shim. *In vitro* skin permeation of Nicotine from proliposomes. J Control Release, 1997; 49: 177-84.
11. Deo MR, Sant VP, Parekh SR, Khopade UV. Proliposome-Based transdermal delivery of Levenogestral. J Biomater Appl, 1997; 12(1): 77-88.
12. More BH, Sakharwade, Tembhurne SV, Sakarkar DM. Evaluation for skin irritancy testing of developed formulations containing extract of Butea monosperma for its topical application. Int J Toxicol Appl Pharmacol, 2013; 3(1): 10-13.
13. Ashok Raj R, Saravana Kumar, Gandhimathi. Hypoglycemic and hypolipidemic activity of Wrightia Tinctoria L. in Alloxan induced diabetes in Albino Wistar rats. Pharmacologyonline, 2009; 3: 550-59.