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Development of Proliopsomal Gel Containing Glipizide for Better Anti Diabetic Effect

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ABSTRACT

The present investigation was aimed to develop Glipizide loadedproliopsomal carrier system for Transdermal drug delivery which, deliver the drug effectively over an extended period of time to improve the anti diabetic effect of the loaded drug. Glipizide loaded proliposomal gel was prepared by coacervation-phase separation method using different combinations of drug, sorbitol with cholesterol and lecithin (GF1 – GF6). Proliposome formulations were characterized for physical appearance, pH, vesicle size, entrapment efficiency, drug content uniformity, surface morphology, zeta potential analysis, *in-vitro* drug release, skin irritation test, and hypoglycemic activity. Among the different formulation, GF2 formulation showed more entrapment efficiency and drug content compared to all other formulations. The optimized formulation GF4 showed maximum reduction in blood glucose level 101.83 ± 0.983at 24 hrs as compared to oral drug. Proliposomal gel (PLG1) showed no skin irritation and delivered the Glipizide in a controlled manner as compared to conventional dosage form, as evidenced by a significant decrease in blood glucose level in diabetes rats. Thus proliposomal gel will be suitable drug delivery system for Glipizide due to ease of preparation and incorporation of less number of excipients.

Keywords: Proliposomes, Glipizide, *in vitro* release, hypoglycemic activity, Transdermal delivery.

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INTRODUCTION

Concurrent with the spread of the western lifestyle, the prevalence of all types of diabetes is on the rise in the world's population. The number of diabetes is increasing by 4-5% per year with an estimated 40-45% of individuals over the age of 65 years having either type II diabetes or impaired glucose tolerance. According to the recent dates from WHO, approximately 150 million people have diabetes worldwide, ad that this number may well double by the year 2025 due to population growth, ageing, unhealthy diets, obesity and sedentary lifestyles.[1,2]

Now a day's most commonly prescribed anti diabetic drugs for treatment of type 2 diabetes (NIDDM) are given by oral route of administration. Most of the oral anti diabetic drug shows poor bioavailability due solubility problem and extensive first-pass metabolism, and also the absorption is dependent on formulation type and food. They also produce adverse effect like gastric disturbances and toxicity due to chronic use of this drug. Hence there is a need for alternative delivery systems to administer the drug by the safer route and to improve bioavailability as well improve the patient compliance by many folds. To improve such characters transdermal drug delivery system was emerged. Transdermal systems are ideally suited for diseases that demand chronic treatment. Hence, anti-diabetic agents of both therapeutic and prophylactic usage can be subjected to transdermal investigation[3].

Recently colloidal systems of nano-size range are constantly being developed as drug delivery carriers for diverse application, such as the oral, transdermal, parenteral and ocular delivery. Among the various colloidal system developed, the liposomes have gained much interest and often been considered to be a potential option but, posing long-term stability issue. 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. When proliposomes are applied to mucosal membranes, they are expected to form liposome upon hydration by mucosal fluids. The resulting liposome may act as a sustained release dosage form of loaded drugs[4, 5].Liposome formed on hydration has the ability to modulate diffusion across the skin. They do so by fusing with the skin surfaces and establishing concentration gradient of the intercalated drug across the skin. Thus they enhance skin permeation. Also, the vesicle intercalation into the intracellular lipid layers of the skin results in fluidization and disorganization of the regular skin structure, obviating the barrier function of the stratum corneum[6, 7, 8].

Glipizide is an oral blood- glucose- lowering drug of the sulfonyl urea class. Glipizide has been in extensive use to treat non-insulin dependent diabetes mellitus and acts by increasing the release of

endogenous insulin as well as its peripheral effectiveness[9]. It has been associated with sever and sometimes fatal hypoglycemia and gastric disturbances like nausea, vomiting, heart burn, anorexia and increased appetite after oral therapy. The Glipizide is having half-life of about 3-5 hrs undergoes first pass metabolism. Hence, it is suitable drug candidates to design into an effective transdermal system, which can eliminates its first pass metabolism, ensure more uniform plasma levels, reduces side effects like gastric irritation and improve patient compliance.[10]

Therefore, the specific objective of this study is to find out the feasibility of proliposomal gel formulation of Glipizide an oral antidiabetic agents and its evaluation for improved transdermal delivery.

MATERIALS AND METHOD

Glipizide was gifted from Biocon Limited, Bangalore (India), soya lecithin was gifted from Pharma Sonic Biochem Extractions Ltd., Indore (India).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 Glipizideproliposome

Glipizide loaded proliopsomal carrier system was prepared by using thin film deposition on carrier method using vacuum rotary evaporator**.** Sorbitol as carrier and different ratio of lecithin and cholesterol have been used in the formulation(GF1- GF6). 1 g of sorbitol (sieved with sieve no. 100 meshes) was placed in round bottomed flask at 60 - 70 ℃ and rotated at 115 rpm under vacuum for 30 min till complete drying. Drug, lecithin and cholesterol were dissolved in mixture of chloroform and methanol in the ratio of 8:2 (v/v) 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. From the different formulation the optimized formulation was incorporated in 1% w/v Carbapol gel for further study (PLG1)[11].

Table 1: Formulation design for the preparation Glipizide loadedproliposomes

Formulation Code	Drug (mg)	Sorbitol (mg)	Soya lecithin(mg)	Cholesterol (mg)
GF1	100	1000	50	50
GF2	100	1000	100	50
GF3	100	1000	150	50

GF4	100	1000	200	50
GF5	100	1000	100	25
GF6	100	1000	200	75

Evaluation of Glipizideproliposomes

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[12, 13].

***In Vitro* drug release studies**

The Diffusion cell with cellophane was used for in *vitro* drug release study. The dialysis tube was suspended in 500 ml beaker,containing 250 ml phosphate buffer pH 7.4. The Medium was stirred at 100 rpm withthe help of magnetic stirrer at 37 ± 0.5 ℃. The samples were withdrawn at suitable time interval(at 1, 2, 4, 6, 8, 12, 16, 20 and 24 hr) and maintained sink condition. The samples (5 ml) wereanalyzed by UV spectrophotometer at λmax 276 nm andcumulative % of drug released was calculatedand plotted against time (t). The rate and release mechanism of Glipizide from the prepared proliposomes were analyzed by fitting the releasedata in to various kinetic models [14].

Skin irritation test:

The Wister albino rats of either sex weighing 150 – 20 g were taken for skin irritation studies and the intact skin was used for this study. The animal were observed and evaluated for any sign of erythematic or edema for aperiod of 7 days after application of proliposomal l gel on the backarea (approximately 6 cm^2 hair removed area) [15].

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 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/RP/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 [16].

Preparation of animals for studies:

Hairs on the backside (interscapular region) of the rats were removed with adepilatory cream and treatment was provided topically on hair removed area. The animals were divided into 4 groups (n=6) of diabetes rats and 1 group (n=6) of normal rats. The rats astreated as following.

Group I - Normal control rats received 0.9 % saline solution

Group II - Diabetic control rats received 0.9 % saline solution

Group III -. Hyperglycemic rats received oral dose 0.2 mg/kgGlipizide suspension

Group IV - Hyperglycemic rats received proliposomal gel contains Glipizide (PLG1).

The blood was collected by pricking the rat's tail at appropriate time intervalfor 24 hrs and blood glucose level was measured immediately by using digitalglucometer.

RESULTS AND DISCUSSION

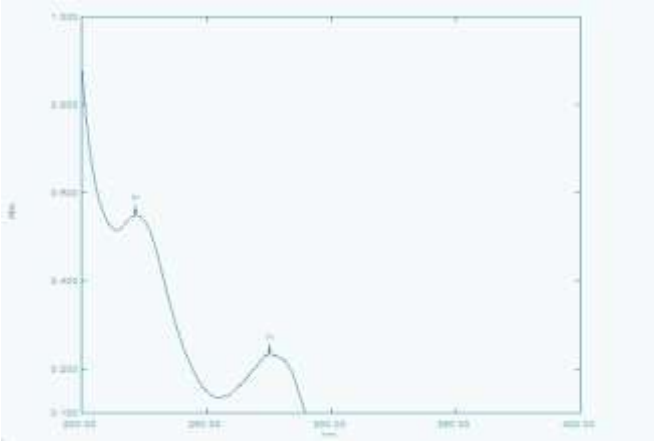

Figure 1: Determination of λmax of the Glipizide

The λmax of the Glipizide in phosphate buffer pH 7.4 was found to be 276 nm and the curve was shown in Figure.1

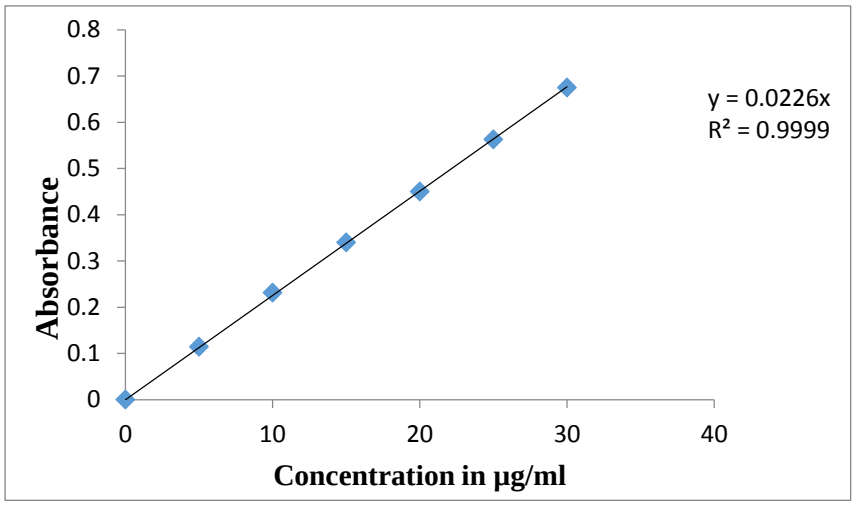

Figure 2: Standard calibration curve of Glipizide at λmax 276 nm

Glipizide obeys the Beer's law in concentration range of 5-30 µg/ml in phosphate buffer pH 7.4 with regression of coefficient of 0.9999and slope 0.0226. Calibration curve was shown in Figure.2

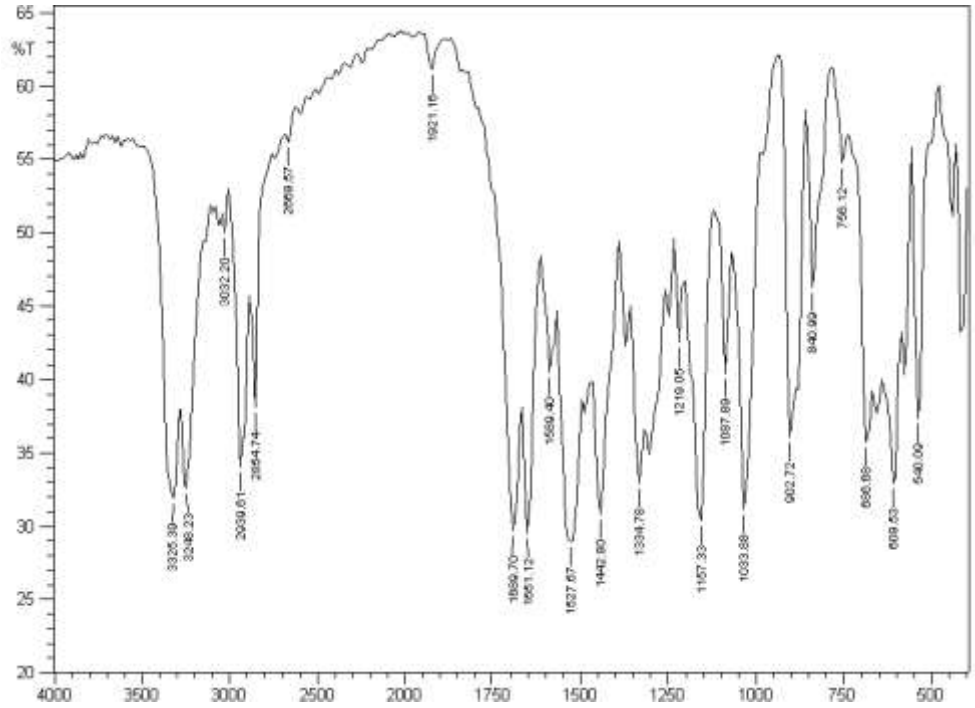

Figure 3: FT-IR Spectroscopy of Glipizide

FTIR spectra of pure Glipizide showed sharp characteristic peaks at 3248.23(N-H Stretching), 2939.619 cm-^{1}(C-H Stretching), 1689.70 cm-^{1} (C=O Stretching), 1442.80 cm-^{1} (C-H Bending), 1157.33 cm-^{1} (S=O Stretching) as shown in Figure.3

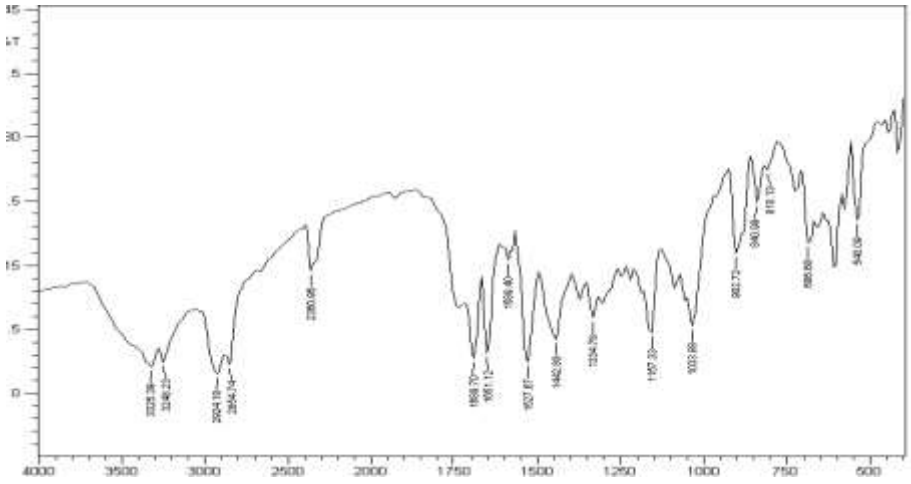

Figure 4: FT-IR Spectroscopy of physical mixture of Glipizide +Sorbitol +Soya Lecithin+Cholesterol

FTIR characteristic peaks of pure drug are also observed in the spectra of physical mixture indicating no interaction between the drug and excipients as shown in Figure.4

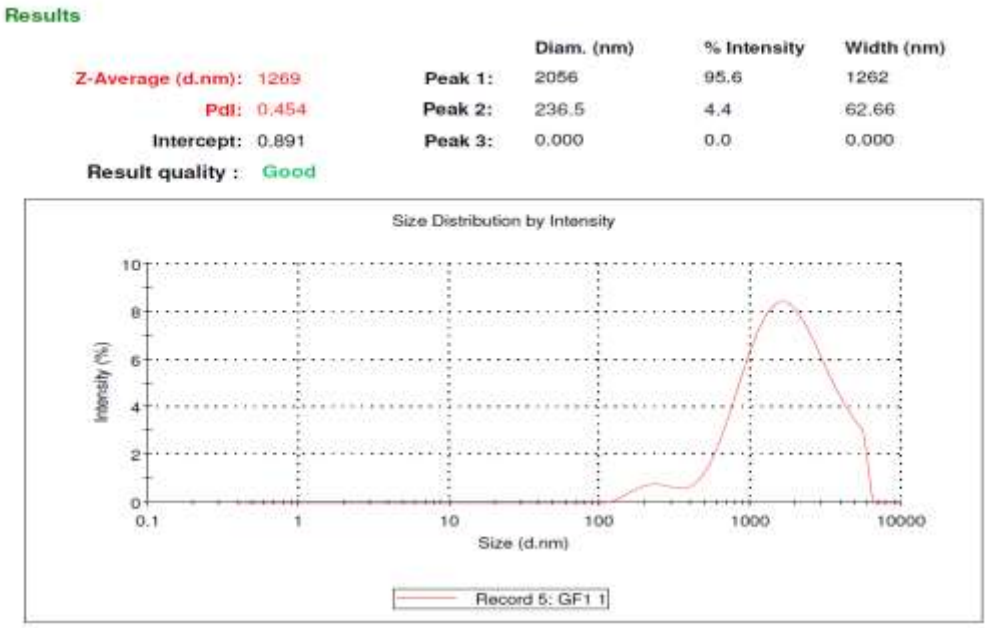

Figure 5: Average particle size of optimized proliposomes formulation GF2

Figure.5 depicted the average vesicle size of GF2 formulation by Malvern particle size analyzer and was found to be 1269 nm with poly dispersity index of 0.454

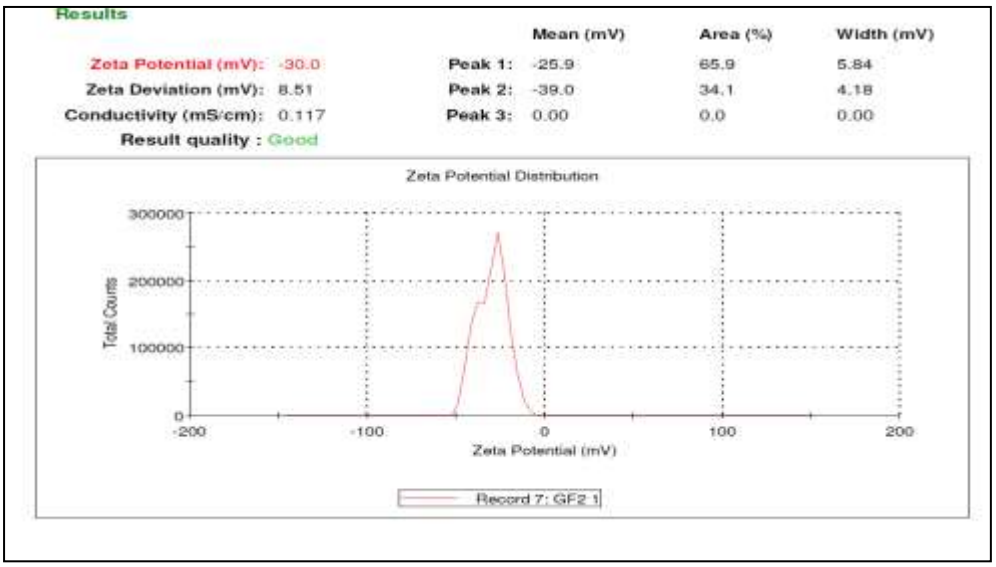

Figure 6: Zeta potential of optimized proliposomes formulation GF2

The mean zeta potential of the optimized formulation were measured as - 30 mV by Malvern zeta analyzer and the result is depicted in figure 6

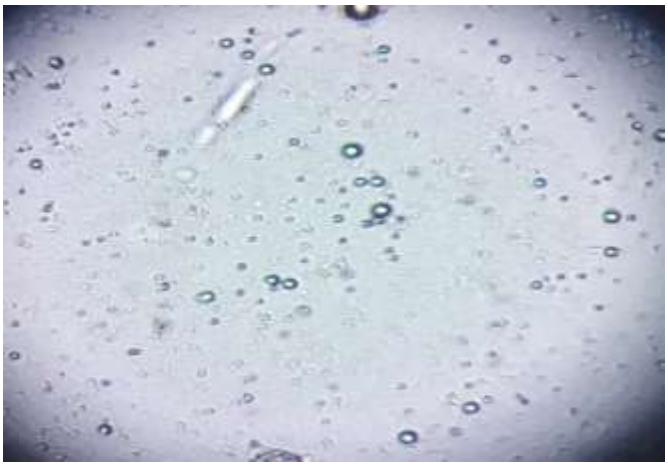

Figure7:Microphotographs of proliposomal gel formulation GF2

Figure8: Microphotographs of proliposomal gel formulation GF5

The spontaneous formation of vesicles after hydration of proliposomal formulation were observed by optical microscope and presented in Figure 7 and 8.

Table 2: Drug content and % Entrapment efficiency of proliposomes formulations GF1 – GF6

Formulation code	% Drug content	%Entrapment efficiency
GF1	83.21	90.12
GF2	95.26	95.70
GF3	92.31	96.21
GF4	87.12	96.73
GF5	96.76	97.77
GF6	89.72	80.20

The percent drug content and entrapment efficiency of prepared proliposomal formulation were varied according to the soya lecithine and cholesterol content.

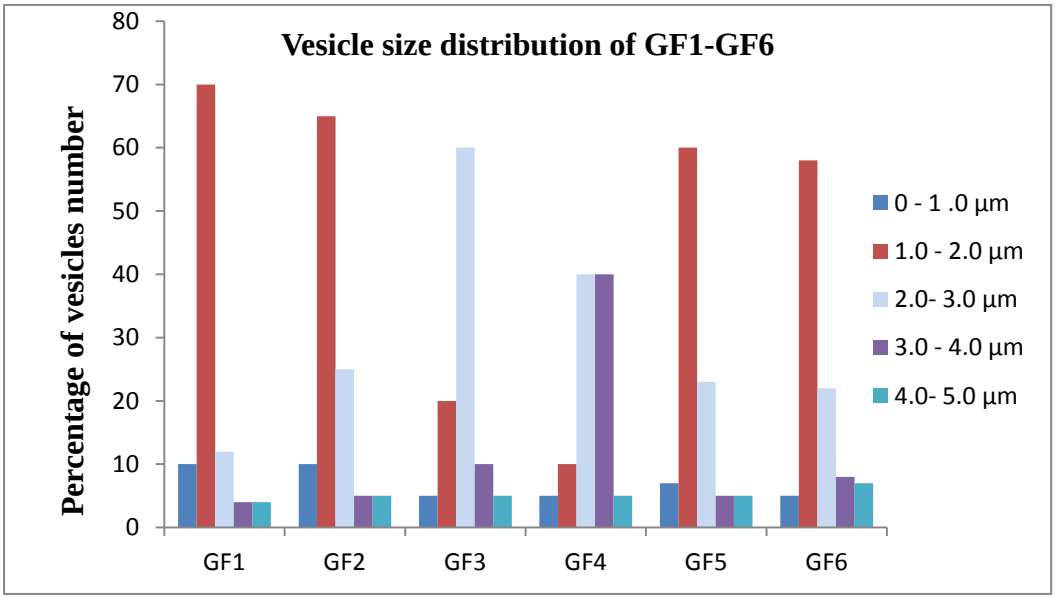

Figure9: Vesicle size distribution for proliposomes formulation GF1-GF6

About 300 vesicles from each formulation were measured for the particle size determination using optical microscope and vesicle size distribution was presented in percentage of vesicle number in each size range and depicted in Figure 9

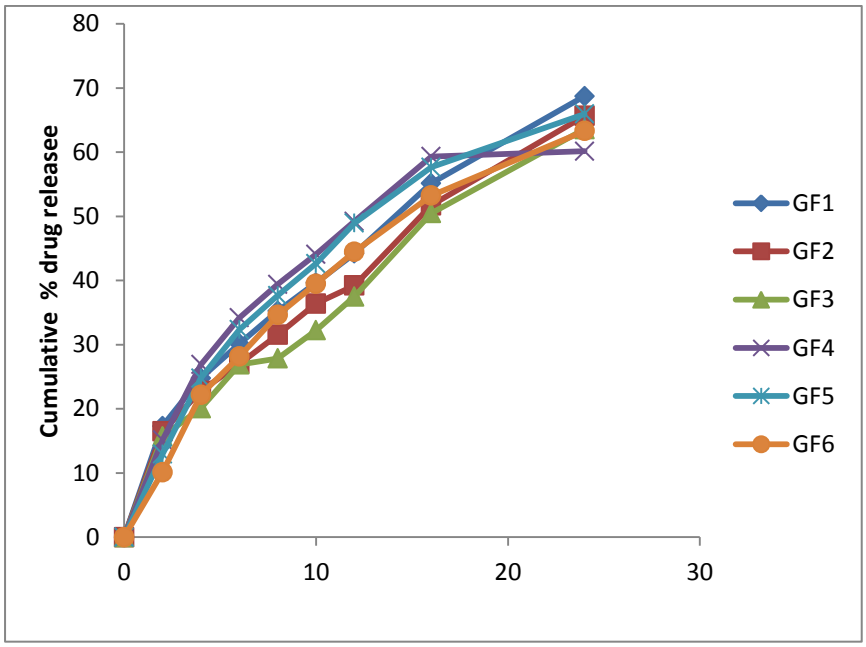

Figure 10: % Cumulative drug release of proliposomes formulation from GF1-GF6

The release of Glipizide from proliposomal formulation varied according to the ratio of soyalecithin. The progressive decrease in the amount of drug diffused through a dialysis membrane from formulations GF1 to GF4 attributed to increased ratio of soyalecithin. The difference in drug release from GF5 – GF6 might be the differences in cholesterol content

Table 3: Release kinetic model fit data

Formulation code	Zero order	First order	Higuchi plot	Peppas plot	
				r^2	'n'
GF1	0.940	0.991	0.990	0.781	1.102
GF2	0.953	0.987	0.979	0.786	1.083
GF3	0.959	0.982	0.964	0.795	1.076
GF4	0.905	0.983	0.993	0.798	1.141
GF5	0.930	0.994	0.989	0.828	1.165
GF6	0.922	0.983	0.983	0.854	1.167

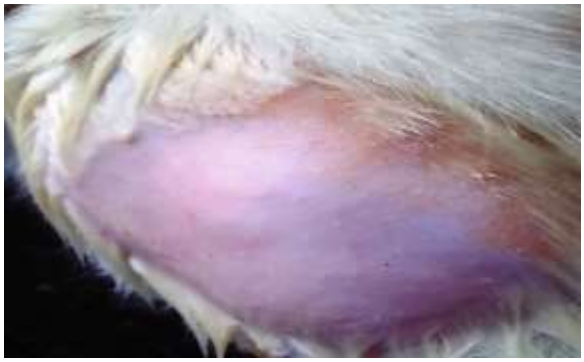

Figure11: Rat skin for observation of erythma and edema (After 7 days) for proliposomal gel formulation PLG1

Table 4: Hypoglycemic activity of Glipizide proliposomal gel formulation PLG1

Time(hr)	Reduction in blood glucose level in mg/dl (mean ± SD, n=6)			
	Group-I	Group-II	Group-III	GroupIV*
0	95.66 ± 0.516	272.00 ± 0.516	265.16 ± 0.752	265.83 ± 0.752
2	93.66 ± 0.816	261.33 ± 0.816	210.50 ± 0.836	225.16 ± 0.752
4	94.33 ± 0.516	265.50 ± 0.547	160.83 ± 0.983	199 .33 ± 0.816
8	95.50 ± 0.547	255.16 ± 0.752	115.16 ± 0.752	181.00 ± 0.894
10	97.66 ± 0.516	251.00 ± 1.032	151.66 ± 1.032	158.66 ± 0.816
12	93.33 ± 0.516	258.00 ± 0.816	201.16 ± 1.169	131.16 ± 0.752
24	91.33 ± 0.516	253.50 ± 1.041	241.50± 1.048	101.83 ± 0.983

*P<0.0001, all values are expressed mean ±SD. Significance was performed by ANOVA using Graph pad prism 7.01.

Group-I Normal control; Group-II = Diabetic control Group-III = oral route, Group-IV = PLG1 (Topical),

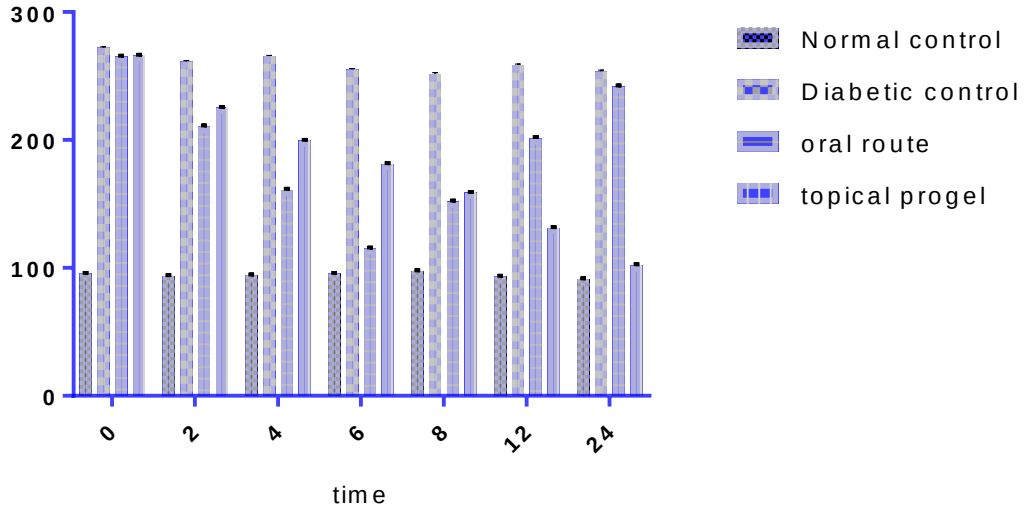

Figure12: Reduction of Blood glucose level after oral and proliposomal gel application of Glipizide in diabetic induced rat.

DISCUSSION

Glipizide showed λmax at 276 nm (Figure. 1) in phosphate buffer pH 7.4 and obeys the Beer's law in concentration range of 5-30 µg/ml in phosphate buffer pH 7.4 with regression of coefficient of r^2 0.9999 and slope of 0.0226 as shown in Figure.2.

The compatibility of the drug and other excipients were confirmed by FTIR studies.The characteristic peaks of pure drug are also observed in the spectra of physical mixture indicating no modification for interaction between the drug and excipients. FTIR graphs are showed in Figure 3 and 4.

The vesicles were observed by optical microscopy and sizes were measured for 300 vesicles and percentage of vesicle size distribution of different sizes were analyzed and results were depicted in Figure 9. On hydration with pH 7.4 buffer solution the proliposomes powders were quickly form the vesicles as shown in microphotographs of GF2 and GF5 in Figure 7 and 8. . In GF1 formulation the maximum percentage (70%) of vesicles lies in the size range of 1.0 – 2.0 µm. When we increase the concentration of soya lecithin the % vesicle size distribution was observed to the increased size of vesicles. In GF2 formulation we have observed nearly 25 % of vesicle were in the range of 2.0 – 3.0 µm, further increase in the soya lecithin concentration in GF3 formulation it was shown that 60 % of vesicles were in the size range of 2.0 – 3.0 µm and for GF4 40 % of vesicle in size range of 2.0 – 3.0µmand another 40 % of vesicles in the size range of 3.0 – 4.0 µm. This result clearly showed that on increasing the soya lecithin concentration the vesicles size were increased. But in formulation GF5 and GF6 we kept the soya lecithin concentration constantly and changing the ratio of cholesterol. From the results we observed that there is no much difference in the size of vesicles but the integrity of vesicle have been increased by increasing the cholesterol. Further the average particle size for the optimized formulation GF2 are shown in figure.5 and it was found to be 1269 nm.

All the formulation were showed better entrapment efficiency of more than 90 % as shown in table 2 , but in GF6 formulation showed 80.40 % due to highly integrated vesicles.

The % drug content of formulation GF2 and GF5 showed maximum drug content up to 95.26% and 96.76%, respectively with maximum entrapment efficiency of 95.70 % and 96.73% respectively 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 GF2 were measured by Malvern and it shows the value of -30.0 mV which indicates that they are sufficient to be stable. The results are shown in Figure 6.

The *in vitro* drug release of Glipizide in proliposome through dialysis membrane for all the formulation GF1 – GF6 are shown in Figure 10. From the results we observed that increasing the ratio of soya lecithin the diffusion of drug from the proliposomes gradually decreased as the vesicle size have been increased. The drug release for the formulation after 24 hrs showed 68.73 % for GF1 and 65.65 %, 63.6%, 60.15% respectively for the formulations GF2, GF3 and GF4. The effect of cholesterol content have been studied in the formulation GF5 and GF6, which showed that decreasing the cholesterol content the drug release was more 65.95 % for GF5 as compared to higher content of cholesterol *i.e.*, 63.32 % for GF6. This may be attributed that increasing the cholesterol content may increase the integrity of the vesicle surface and slowdown the release of

drug from the formulation. The drug release were fitted to different kinetic model is given in Table 3. From the results it was observed that all the formulation followed 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.

The skin irritation study of proliposomal gel formulations have been carried out for 7 days to observe any irritation erythema. But the results showed no irritation and erythema after application of proliposomal gel as shown in Figure 11.

The results of hypoglycemic activity of Glipizide proliposomalgel(PLG1) with normal control, diabetic control and oral suspension were showed in Table. 4 and Figure 12. In group III which receives oral glipizide showed a maximum reduction in blood glucose level within 4 hrs and the blood glucose level have been increased after 10 hrs. Whereas, for the proliposomal gel PLG1 (groupIV) the blood glucose level was reduced in a controlled manner and observed blood glucose level reduction in group IV in gradual and controlled manner and showed maximum reduction of blood glucose level to 101.83 ± 0.983 at the end of 24 hrs. Whereas normal control and diabetic control didn't show any reduction in blood glucose level (no hypoglycemic effect). From these results it was concluded that the application of topical proliposomal gel offers better therapeutic effect of loaded drug and produced controlled systemic effect.

CONCLUSION

Glipizide has been in extensive use to treat non-insulin dependent diabetes mellitus and acts by increasing the release of endogenous insulin as well as its peripheral effectiveness. It has been associated with sever and sometimes fatal hypoglycemia and gastric disturbances like nausea, vomiting, heart burn, and increased appetite after oral therapy, compliance problem can arise. Hence, Glipizide is a suitable drug candidate for design into an effective transdermal delivery system which could eliminates its first pass metabolism, ensure more uniform plasma levels, reduces side effects like gastric irritation and hence aid in patient compliance. From the study of the hypoglycemic activity , it was concluded that the topical application of proliposomal gel containing Glipizide are more effective as compared to oral formulation because it provide reduction in glucose level with controlled manner up to 24 hrs avoiding other side effects associated with Glipizide.

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