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FORMULATION AND EVALUATION OF PROLIPOSOMAL GEL CONTAINING METFORMIN HYDROCHLORIDE USING MANNITOL AS A WATER SOLUBLE CARRIER

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ABSTRACT

The aim of the present study was to develop and characterized a vesicular drug carrier system (proliposome) for topical delivery of Metformin hydrochloride to overcome the problems related with oral route. Proliposomes of Metformin hydrochloride were prepared by thin film hydration technique by varying the composition drug, manitol, soya lecithin and cholesterol. Proliposome formulations were characterized for compatibility, Vesicle size, %Drug content, %Entrapment efficiency, Surface morphology, Surface charge, *invitro* drug release and stability studies. The proliposomal gel was prepared for optimized proliposomal formulation F4 by incorporated into 1% Carbopol gel. The *in vitro* drug release and *in vivo* skin irritation study and hypoglycemic activity were carried out for the gel F4-G1. Drug and physical mixture were characterized by FTIR, the result of IR 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. Proliposomal gel F4-G1 was proved nonirritant and showed better stability, more hypoglycemic effect as compared to oral formulation because it provide reduction in blood glucose level with controlled manner upto 24 hrs. Hence, Proliposomes drug delivery system was better choice for sustained release of drug through topical drug delivery.

Keywords: Proliposomes, Liposomes, Proliposomal gel, Sustained release, Hypoglycaemic activity.

INTRODUCTION

Proliposomes are novel generation of carrier mediated drug delivery system having several advantages over conventional liposomes. It has shown better stability and ease of sterilization on large scale by preventing drug over loading. Maximum amount of drug encapsulation helps in more penetration of drug and producing a sustain release effect at the site of administration [1].

Proliposome are simply soluble particles covered with liposome precursors which, when dissolved in water, will produce liposomes. Payne et al., (1986a, 1986b) originally developed this method [2]. Being available in dry powder form, they are easy to distribute, transfer, measure and store making it a versatile system. Liposomes can either be formed *in vivo* under the influence of physiological fluids or can be formed *in vitro* prior to administration using a suitable hydrating fluid. The liposomes formed on reconstitution are similar to conventional liposomes sand more uniform in size [3].

Diabetes mellitus is group of metabolic disorders characterized by chronic hyperglycemia. In recent years, developing nations have witnessed an explosive increase in the prevalence of diabetes mellitus predominantly related to life science changes and the resulting surge in obesity. The metabolic consequences of prolonged hyperglycemia and dyslipidemia including accelerated atherosclerosis, chronic kidney disease pose enormous burden of patients with diabetes mellitus and on the public health system. An estimated 20.8 million people currently has diabetes, of these, 6.2 million or about 1/3rd. were undiagnosed. In 2005 alone, over 1.5 million new cases in adults were diagnosed. Globally the prevalence of diabetes for all ages is estimated to be 2.8% in 2000 and projected to 4.4% by 2030. The centers for disease control and prevention predicts the national incidence of diabetes will rise by 37.5% by the year 2025 [4].

Metformin hydrochloride, an oral anti - diabetic

drug frequently used as first line drug of choice in treatment of type 2 diabetes, particularly in overweight and obese people and those with normal kidney function. Metformin hydrochloride is anti-hyperglycemic drug and it does not increase insulin release in the pancreas. Metformin hydrochloride reduces glucose levels primarily by decreasing hepatic glucose production and by increasing insulin action in muscle and fat. Metformin hydrochloride is absorbed mainly from the small intestine and does not bind to plasma proteins. Metformin hydrochloride is safe and not teratogenic in many of species studied. Metformin hydrochloride however has many gastro intestinal side effects including nausea, vomiting, anorexia and diarrhoea and rapid first pass metabolism. The transdermal Metformin hydrochloride allows delivery of drug through the skin and bypassing the digestive system [5].

This route has many advantages from orally dosed Metformin hydrochloride;

They are,

1. Avoids gastrointestinal side effects by avoiding the digestive system.
2. Potentially reduces cost through fewer side effects.
3. Smaller dose is required when metformin in penetrated through skin.
4. Improve the bioavailability as well improve the patient compliance.

The main objective of the study is to formulate and evaluate Metformin hydrochloride proliposomal gel in order to increase bioavailability and reduce side effects by achieving transdermal drug delivery.

MATERIALS AND METHOD

Materials

Metformin hydrochloride and Mannitol was gifted from Medreich Limited, Karnataka, (India), Soya lecithin was gifted from Pharma Sonic Biochem Extractions Ltd., Indore (India). Cholesterol, Carbopol 934, Triethanolamine, Methyl paraben, Alloxan and other solvent like Chloroform and Methanol purchased from S d fine chem limited, Mumbai (India).

Experimental model

Male Wister albino rats, 150-200 gm weight, were selected for *in vivo* studies. All animals were fed with a standard laboratory diet and water. They were housed in a specific room at a temperature of 20-25 °C and 50 ± 5% relative humidity under a 12 hrs dark/ light cycle and acclimatized for 1 week before the start of experiment. All experimental procedures involving animals were approved by the Institutional of Animal Ethical Committee (IAEC) of Bharathi College of Pharmacy.

PREFORMULATION STUDIES

Determination of Solubility

Metformin hydrochloride is white, crystalline powder. Solubility was conducted using different solvents

Melting point determination

Capillary method was used for the determination of melting point of Metformin hydrochloride. A few quantity of Metformin hydrochloride is taken and placed in a thin walled capillary tube about 10-15 cm long and 1mm inside diameter and closed at one end. The capillary which contains the sample and a thermometer are then suspended into an oil bath containing liquid paraffin. So they can be heated slowly and evenly. The temperature range over which the sample is observed to melt is taken as the melting point.

Drug-Excipient interactions studies by FTIR

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

METHODS

Preparation of Metformin hydrochloride proliposome

Metformin hydrochloride proliposome was prepared by using thin film deposition on carrier method using vacuum rotary evaporator (Buchi Corporation, Switzerland). Optimization of proliposome formulation was done by preparing varying concentration of mannitol, lecithin and cholesterol. 1 gm of mannitol powder (sieved with 100 meshes) was placed in round bottomed flask at 60-70°C and 115 rpm under vacuum 30 minutes for complete drying. Metformin hydrochloride 100 mg and lecithin, cholesterol were dissolved in mixture of chloroform and methanol in the ratio of 8:2 (v/v) for various formulation as shown in Table 1. Initially 0.5 ml aliquot of organic solvent was introduced into round bottomed flask at 37°C and rotated, after complete drying second aliquot 0.5ml 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 [6-7].

Preparation of Metformin Hydrochloride proliposomal gel

Proliposomal gel was prepared for the optimized formulation, by cold mechanical method as per the composition given in [Table. 2]. Required quantity of polymer was weighed and it was sprinkled slowly on surface of purified water with continuously stirred by mechanical stirrer and allowed swell for 24 hrs to obtain 1% gel [7].

Accurately weighed proliposomes was dissolved in 10 ml of methanol and centrifuged at 25000 rpm for 60 min to remove the untrapped drug. The supernant was decanted and sediment was incorporated into the gel vehicle. The incorporation of the proliposomes into gels was achieved by slow mechanical mixing at 25 rpm for 10 min. Triethanolamine was added to bring the pH neutral. The final quantity was made up to 5 gm with distilled water.

Evaluation of Metformin hydrochloride proliposomes

Prepared proliposomal formulation was evaluated for the following parameters [8].

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 eyepiece micrometer. About 300 liposomes were measured individually, average was taken and their size distribution range and mean diameter were calculated.

Surface morphology by scanning electron microscopy (SEM)

Surface morphology of the proliposomes will be determined by using a Scanning electron microscope. Proliposomes was coated with Gold-palladium alloy of 120°A Knees on the sample sputter coating unit (Model E5 100 Polaron U.K) and their surface morphological were photographed with Jeol JSM-T330A, Japan scanning electron microscope.

Surface charge

A surface charge of proliposomes was studied by determining the zeta potential of the vesicles formed after hydration of proliposomes. The optimized proliposomes was dissolved in distilled water and made a higher serial dilution 1000 X until a clear solution is obtained. Sample was analyzed for determining the Zeta potential using PALS Zeta potential Analyzer (Brookhaven Instruments Corp.)

Drug content

100 mg of proliposomes formulation was weighed and vesicles were lysed with 25 ml of methanol solution by sonication for 15 min. The clear solution after suitable dilution was measured by U.V spectrophotometer (Shimadzu UV-1800, Japan) against blank at λ_{\max} 232 nm and the %drug content was calculated.

Entrapment efficiency

The entrapment efficiency (EE %) of Metformin hydrochloride in the reconstituted liposomes was determined after hydration of proliposomes with distilled water. 10 ml of distilled water was added to the proliposome containing equivalent to 10 mg of drug and the mixture was shaken manually for 2 min. For the separation of untrapped Metformin hydrochloride, the liposomal suspension was subjected to centrifugation on a cooling ultracentrifuge (REMI instrument, Mumbai) at 25000 rpm for 1 hr. The clear supernatant was separated and amount of untrapped drug was determined by UV spectrophotometer at 232 nm.

The % entrapment was determined by following formula:
 Percentage entrapment = $\frac{\text{Total drug content} - \text{Free drug content}}{\text{Total drug content}} \times 100$.

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 10 mg Metformin hydrochloride). The dialysis tube was suspended in 250 ml beaker, containing 100 ml phosphate buffer pH 7.4. The solution was stirred at 200 rpm with the help of magnetic stirrer at 37 ± 0.5 °C. 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 and 24 hrs). The dissolution medium was replaced with same amount of fresh phosphate buffer pH 7.4 solutions to maintain the volume 100 ml throughout the experiment. The drug content in the withdrawn samples (1 ml) were analyzed by UV spectrophotometer at λ_{\max} 232 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 Metformin hydrochloride from the prepared proliposomes were analyzed by fitting the release data in to various kinetic models.

Stability studies

Intermediate stability testing studies was performed for 6 months as per ICH guidelines. The optimized formulation was kept at 30 ± 2 °C and $65 \pm 5\%$ RH in stability chamber (Thermo lab India). % entrapment, vesicle size and drug release were fixed as physical parameters for stability testing.

Evaluation of Metformin hydrochloride gel

Physical examination

The physical appearance of color, consistency texture and greasiness these all features were done for proliposomal gel formulation [9-10].

Measurement of pH

The pH of various gel formulations was determined by using digital pH meter (Equiptronics EQ-610). The electrode first calibrated with pH 4.0 and pH 7.0 solution then measurement of pH of each formulation was done.

Viscosity and Rheological properties

Brookfield digital viscometer (DV-I+, Brookfield Engineering Laboratory, INC., USA) was used to measure the viscosity (in cps) of the prepared gel formulation.

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 λ_{\max} 232 nm and the drug content was calculated.

In vitro release studies of proliposomal gel

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 $37\pm 0.5^\circ\text{C}$ with stirring at 500 rpm throughout the experiment. At fixed time interval, 1ml of aliquots was withdrawn for every 1, 2, 4, 6, 8, 12 and 24 hrs from receiver compartment through side tube and after suitable dilution analyzed by UV spectrophotometer at λ_{max} 232 nm.

In vivo studies

Skin irritation test

Four 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 [11].

Hypoglycemic activity

Induction of diabetes

Rats were fasted for 24 hrs and blood glucose level of each group was assessed to obtain the fasting blood glucose levels. Alloxan at dose of 100 mg/kg body weight in PBS pH 7.4 was administered by intraperitoneal route to each rat and 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 [12-13].

Preparation of animals for studies

Hairs on the backside 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 2 groups (n=4) of diabetes rats. The rats as treated as following.

Group I- Metformin hydrochloride oral Administration contain 2 mg drug in PBS.

Group II- Proliposomal gel contains Metformin Hydrochloride.

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

Stability studies for proliposomal gel

Intermediate stability testing studies was performed for 6 months as per ICH guidelines. The optimized gel formulation was kept at $30\pm 2^\circ\text{C}$ and $65\pm 5\%$ RH in stability chamber. Drug content, pH and drug release were fixed as physical parameters for stability testing.

RESULTS AND DISCUSSION

Metformin hydrochloride is a white, crystalline, freely soluble in water, methanol and soluble in ethyl alcohol and insoluble in chloroform. Melting point was

determined by capillary method and it was found to be 223°C , which complied with IP standards, thus indicating the purity of drug. FTIR spectra of pure Metformin hydrochloride showed sharp characteristic peaks at 3371.68, 3292.60, 3173.01, 1629.90, 1629.90, 1477.52, 1037.74, 935.51cm^{-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.

Proliposomes were prepared using film deposition on carrier method using vacuum rotary evaporator. The result showed in Table.4, we observed that, increase in the concentration of soya lecithin and cholesterol vesicle size, %Drug content and % Entrapment efficiency was found to be increased. The surface morphology was studied by Scanning electron microscopy (SEM). The SEM photographs of optimized proliposomes formulation F4 as shown in Fig. 3. 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. Zeta potential of optimized formulation F4 was found to be -40.92 mV, which indicates that the formulation is good to be stable.

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 78.52 % which was lower among the formulations F1 to F4 and showed in Fig.4. Stability studies were carried out for the most satisfactory formulation F4 at $30\pm 2^\circ\text{C}$ and $65\pm 5\%$ 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 polymers and gel was prepared. The prepared proliposomal gel formulation was evaluated for the following parameters.

Physical appearance

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

pH measurement

The prepared Metformin hydrochloride 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 11247 cps.

Drug content

The prepared Metformin hydrochloride proliposomal gel was subjected to drug content uniformity and it was found to be 4.01 mg/gm which indicated the drug uniformly dispersed throughout the formulation.

In vitro drug release

The releases of drug from these gels were characterized by an initial phase of high release (burst effect). However, as gelation proceeds, the remaining drug was released at a slower rate followed by a second phase of moderate release.

The result of *In vitro* release of Metformin hydrochloride from the gel formulation is given in Fig 5. However, the results clearly show that the gels have ability to retain the drug for prolonged periods. The %CDR of proliposomal gel formulation F4-G1 was found to be 88.58 % and which follows Higuchi model. The 'n' values for all the formulation were found to be less than 0.5. This indicates that the release approximates Fickian diffusion mechanism and this formulation was selected for next *in vivo* and stability studies.

Skin irritation studies

The skin irritation study of proliposomal gel formulations F4-G1 was performed and tabulated in Table. 5. 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.

Hypoglycemic activity

The reduction in blood glucose level in sustained manner after topical administration of Metformin hydrochloride proliposomal gel formulation F4-G1 as showed in Table.6. The pure Metformin hydrochloride suspension was administered orally. A maximum reduction in blood glucose level was observed within 2 hrs. The Metformin hydrochloride proliposomal gel formulation F4-G1 showed significant reduction in blood glucose level but in sustained manner as compared to oral Metformin hydrochloride. From the reported literature, it was well defined that a 25 % reduction in blood glucose levels is considered hypoglycemic effect. Results of present study revealed that topical Metformin hydrochloride proliposomal gel formulation F4-G1 was more effective as compared to conventional formulation because it provide reduction in glucose level with controlled manner up 24 hrs.

Stability studies of proliposomal gel

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.

Table 1. Formulation design for the preparation Metformin Hydrochloride proliposomes

Formulation code	Drug(mg)	Mannitol (mg)	Soya lecithin(mg)	Cholesterol (mg)
F1	100	1000	50	30
F2	100	1000	100	30
F3	100	1000	200	30
F4	100	1000	200	50

Table 2. Formulation design for the preparation Metformin hydrochloride proliposomal gel

S.No	Ingredients	F4-G1
1	Metformin HCl proliposomes (containing pure drug in mg)	20
2	Carbopol 934(mg)	50
3	Methyl paraben(mg)	0.75
4	Distilled water	Up to 5gm

Table 3. Standards for skin irritation study [12]

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 exposure area)	4
Total possible score for irritation	8

Table 4. Vesicle size, % Drug content and % Entrapment efficiency of proliposomes formulations

Formulation code	Average vesicle size in μm	% Drug content	% Entrapment efficiency
F1	6.01	96.29	84.24
F2	6.74	96.59	85.69
F3	7.15	97.95	90.11
F4	7.88	99.02	91.05

Table 5. Reading after Skin irritation study of proliposomal gel formulation F4-G1

Skin responses	Days	Score	
		Rat 1	Rat 2
Erythma 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 (PII)	-	0.16	0.16

Average Primary irritation index of formulation F4-G1 =0.16

Table 6. Hypoglycemic activity of Metformin hydrochloride proliposomal gel formulation F4-G1

Group-I = Oral; Group-II = F4-G1 (Topical)

Time in hrs	%Reduction in blood glucose level (mean \pm SEM n=4)	
	Group-I	Group-II
1	55 \pm 1.32	22 \pm 2.35
2	57 \pm 1.29	29 \pm 2.32
3	44 \pm 2.72	37 \pm 2.29
4	38 \pm 2.32	42 \pm 3.38
6	22 \pm 1.43	39 \pm 3.31
8	-	39 \pm 1.28
12	-	22 \pm 1.34
24	-	09 \pm 2.56

Figure 1. FT-IR Spectroscopy of Metformin hydrochloride

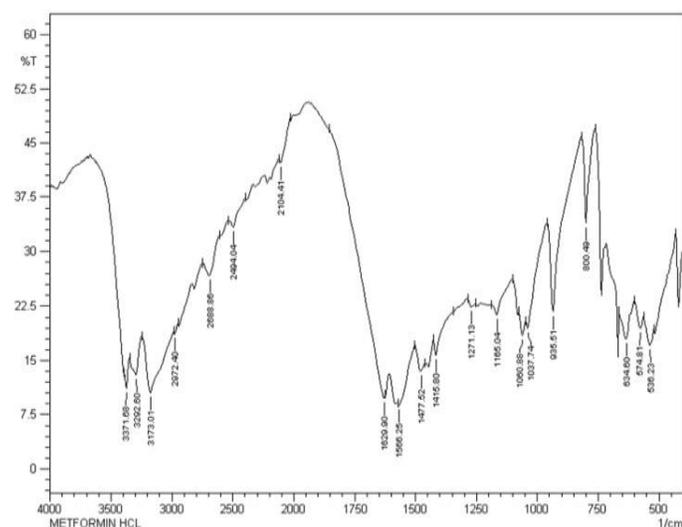


Figure 2. FT-IR Spectroscopy of Metformin hydrochloride+Mannitol+Soya lecithin+Cholesterol

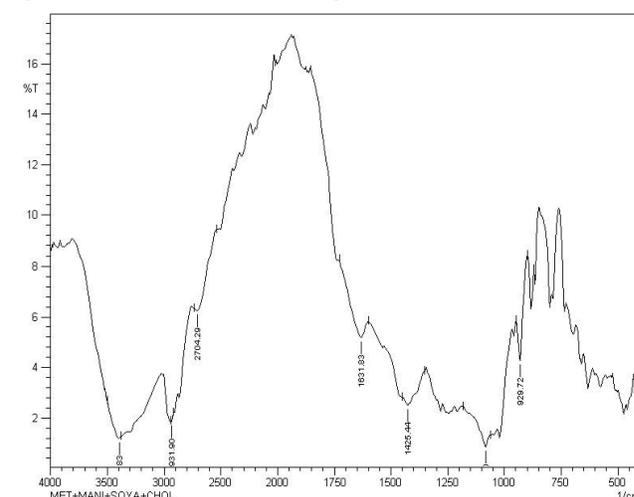


Figure 3. Scanning electron micrograph of proliposomes formulation F4

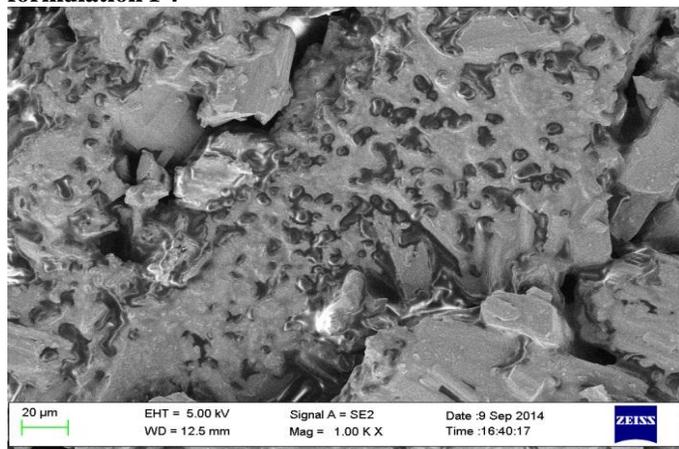


Figure 4. % CDR of proliposomes formulations

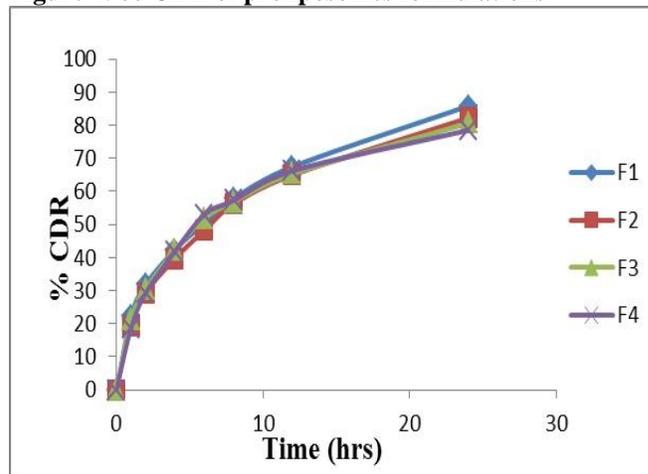
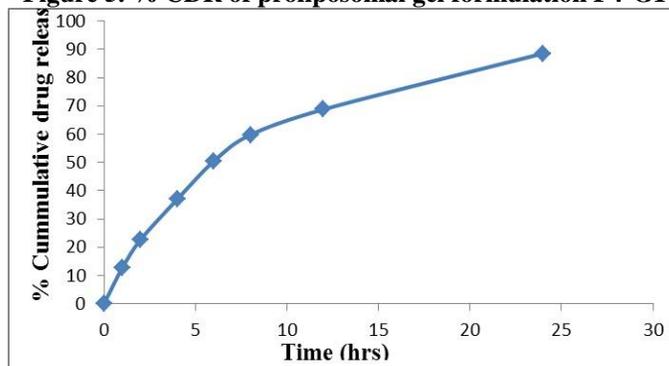


Figure 5. % CDR of proliposomal gel formulation F4-G1



CONCLUSION

Proliposomes exhibited superior stability as compared to liposomes. Proliposomal gel shows no skin irritation and delivered the Metformin hydrochloride in sustained or controlled manner as compared to conventional dosage form, as evidenced by a significant sustained decrease in blood glucose level in diabetes rats. Hence, Proliposomes drug delivery system was better choice for sustained release of drug through topical drug delivery and

topical delivery of Metformin hydrochloride is viable and could improve patient compliance and it may be the most convenient topical formulation for the patient unable to take drug orally.

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REFERENCES

- Malles K, Srinivas C, Nagasree K, Prakash VD. Formulation and Evaluation of Prednisolone Proliposomal Gel for Effective Topical Pharmacotherapy. *IJPDR*, 4(1), 2012, 35-43.
- Ljeoma FU. Synthetic surfactant vesicles niosomes and other non-phospholipid vesicular systems. Publisher: CRC Press, 2000.
- Janga KY, Jukanti R, Velpula A. Bioavailability enhancement of Zaleplon *via* proliposomes: Role of surface charge. *Euro J Pharm and Biopharm*, 80(2), 2012, 347-357.
- Ravi TA, Hemant KS Yadav, Swetha S, Sarat Chandra Prasad M. Preparation and evaluation of transdermal patches of Metformin hydrochloride using natural polymer for sustained release. *Int J Pharm Pharm Sci*, 4(13), 2012, 297-301.
- Bhaves D, Chetan G, Bhat KM. Shivprakash. Estimation and pharmacokinetic of Metformin in human volunteers. *Ind J Pharm Edu Res*, 41(2), 2002, 15-139.
- Hwang BY, Jung BH, Chung SJ, Lee MH, Shim CK. *In vitro* skin permeation of nicotine from proliposomes. *J Control Release*, 49, 1997, 177-184.
- Malles K, Nikitha P, Ranjith K, Pratap C. Piroxicam proliposomal gel -A novel approach for topical delivery. *J Pharm Res*, 5(3), 2012, 1755-1763.

8. Gupta V, Barupal AK, Ramteke S. Formulation development and *in vitro* characterization of proliposomes for topical delivery of Aceclofenac. *Indian J Pharm Sci*, 70, 2008, 768-775.
9. Vure P, Saritha T, Prakash D, Triveni C. Pro-Vesicular (PV)-Based gel for the topical delivery of Naproxen: Preparation, characterization and *in vivo* evaluation. *Asian J Pharm Clin Res*, 7(1), 2014, 195-200.
10. Gupta A, Mishra AK, Singh AK, Gupta A, Bansal P. Formulation and evaluation of topical gel of Diclofenac sodium using different polymers. *Drug Invention Today*, 2(5), 2010, 250-253.
11. Suresh J, Manish G, Anil B and Vimal A. Skin irritation study of intradermal patch of chitosan containing trazodone-HCl on rat skin. *Int J Res Pharm Bio Sci*, 2(3), 2011, 1082-1084.
12. Draize JH, Woodard G and Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J Pharmacolo Exp Ther*, 82, 1994, 377-390.
13. Rakesh KS, Navneet S, Sudha R, Hosakote GS. Solid lipid nanoparticles as a carrier of metformin for transdermal delivery. *Int J of Drug Deliv*, 5, 2013, 137-145.
14. Manish KS, Vikas C, Vandana G and Suman R. Formulation development and characterization of microemulsion for topical delivery of Glipizide. *Der Pharmacia Lettre*, 2(3), 33-42.