



International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: www.ijrpns.com



EFFECT OF SOYALECITHIN CONCENTRATION ON FORMULATION OF ETHOSOMES

K. S. Vijayakumar^{*1}, S. Parthiban¹, G. P. Senthilkumar², A. Vikneswari³

^{1*}Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India.

²Department of Pharmaceutical Chemistry, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India.

³Department of Pharmacy Practice, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India.

ABSTRACT

The aim of the present study was to develop and characterized a vesicular drug carrier system (ethosomes) for topical delivery of Gliclazide to overcome the problems related with oral route. Ethosomes of Gliclazide were prepared by thin film hydration technique by varying the composition drug, soya lecithin and propylene glycol. Ethosomes formulations were characterized for compatibility, Vesicle size, % Drug content, % Entrapment efficiency, Surface morphology, Surface charge, *invitro* drug release and stability studies. The ethosomal gel was prepared for optimized ethosomal formulation F3 by incorporated into 1% Carbopol gel. The *in vitro* drug release and *invivo* skin irritation study and hypoglycemic activity were carried out for the gel F3-G. 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 ethosomes and ethosomal gel are evaluated which showed better results. Ethosomal gel F3-G 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 up to 24 hrs. Hence, ethosomes drug delivery system was better choice for sustained release of drug through topical drug delivery.

KEYWORDS

Ethosomes, Ethosomal gel, Sustained release and Hypoglycaemic activity.

Author for Correspondence:

Vijayakumar K S,
Department of Pharmaceutics,
Bharathi College of Pharmacy, Bharathinagara,
Mandya, Karnataka, India.

Email: ksvijaykumar16@gmail.com

INTRODUCTION

Transdermal administration of drugs is generally limited by the barrier function of the skin. Vesicular systems are one of the most controversial methods for transdermal delivery of active substances Novel drug delivery system (NDDS) has revolutionized the method of medication¹.

Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are composed mainly of phospholipids, (Phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentration of ethanol and water. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum lipids².

Factors affecting physical nature of ethosomes

There are some factors such as hydration temperature, choice of surfactant, nature of membrane, nature of drug, etc., can affect significantly the physical nature of ethosomes.

- Hydration temperature
- Choice of main surfactant
- Nature of drug
- Nature of membrane additives
- Size reduction techniques
- Addition of kinetic energy³

Hence, significant enhanced delivery of through transdermal route could be obtained by using vesicular drug carrier systems-Ethosomes.

Gliclazide is an antidiabetic drug. It is the NIDDM sulfonyl ureas antidiabetic drug, however, the oral administration of the drug is often associated with number of demerits. The major one is low bioavailability and poor water solubility³. Therefore to circumvent drawbacks, administration of drug via transdermal route could be a better option. As transdermal route provides a mean to obtain constant systemic drug levels, high patient compliance because of non-invasive nature. Despite many advantages of the skin as a site of drug delivery, the success of delivery through this route remains limited because the main barrier of the skin is

located in the outermost layer of the skin –the stratum corneum (sc) To overcome the stratum corneum barrier, various mechanisms have been investigated, including use of chemical or physical enhancers such as iontophoresis, sonophoresis, etc. liposomes, niosome, transferosomes and ethosomes also have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier⁴.

This route has many advantages from orally dosed Gliclazide;⁵

They are,

1. It contains non toxic raw materials formulations.
2. Potentially reduces cost through fewer side effects.
3. Smaller dose is required when Gliclazide 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 Gliclazide ethosomal gel in order to increase bioavailability and reduce side effects by achieving transdermal drug delivery.

MATERIAL AND METHOD

MATERIALS

Gliclazide was gifted from Medreich Limited, Karnataka, India. Soya lecithin was gifted from Pharma Sonic Biochem Extractions Ltd., Indore, India. Carbopol 934, Triethanolamine, Methyl paraben, Alloxan and other solvent like Chloroform and Methanol purchased from SD 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

Gliclazide white, crystalline powder. Solubility was conducted using different solvents.

Melting point determination

Capillary method was used for the determination of melting point of Gliclazide. A few quantity of Gliclazide 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, soya lecithin, and the physical mixture of drug: soya lecithin in 1:1 ratio were recorded in between 400 to 4000 cm^{-1} by using liquid sampling technique.

METHODS

Preparation of Gliclazide ethosomes

Classic mechanical dispersion containing Soya lecithin (1-4%) and ethanol (10 to 50%). The drug concentration was fixed as 0.5 % W/V. Accurately weighed quantity of soya lecithin was dissolved in chloroform: methanol (3:1) mixture and the organic solvents were removed in the rotary flash evaporator above the lipid transition temperature (55 °C) (at 60 rpm) to form a thin lipid film in the flask. The traces of organic solvents mixture were further removed by maintaining the temperature under reduced pressure for additional 30 mins after the thin film was formed. Then the lipid film was hydrated with different concentration of hydro ethanolic mixture containing Gliclazide (0.5% w/v) in the rotary flash evaporator at 60 rpm for 1hour in the room temperature. The preparation was vortexed followed by sonication at 4 °C in an ice bath using probe sonicator. After sonication the ethosomal formulation was stored in refrigerator (4 °C) for further studies.

Preparation of Gliclazide ethosomal gel⁶

Ethosomal gel was prepared for the optimized formulation, by cold mechanical method as per the composition given in Table No.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.

Accurately weighed ethosomes was dissolved in 10 ml of methanol and centrifuged at 25000 rpm for 60 min to remove the untrapped drug. The supernatant was decanted and sediment was incorporated into the gel vehicle. The incorporation of the ethosomes 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 Gliclazide ethosomes⁷

Prepared ethosomal formulation was evaluated for the following parameters.

Vesicle size analysis

Vesicle size analysis was carried out using an optical microscope with a calibrated eyepiece micrometer. About 300 ethosomes 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 ethosomes was also determined by PALS Zeta potential Analyzer (Brookhaven Instruments Corp.) for the optimized formulation.

Surface morphology by scanning electron microscopy (SEM)

Surface morphology of the ethosomes will be determined by using a Scanning electron microscope. Ethosomes was coated with Gold-palladium alloy of 120°A Knees on the samples putter coating unit (Model E5100Polaron U.K) and their surface morphological was photographed with Jeol JSM-T330A, Japan scanning electron microscope.

Surface charge

A surface charge of ethosomes was studied by determining the zeta potential of the vesicles formed after hydration of ethosomes. The optimized ethosomes 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.(PALS Zeta potential Analyzer, Brookhaven Instruments Corp.)

Drug content

100 mg of ethosomes formulation was weighed and vesicles were lysed with 25 ml of 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} 226 nm and the drug content was calculated.

Entrapment efficiency

The entrapment efficiency (EE %) of Gliclazide in the constituted ethosomes was determined after hydration of ethosomes with distilled water. 10 ml of distilled water was added to the ethosome containing equivalent to 10 mg of drug and the mixture was shaken manually for 2 min. For the separation of untrapped Gliclazide, the ethosomal suspension was subjected to centrifugation on a cooling ultracentrifuge (REMI instrument, Mumbai) at 20000 rpm for 1 hr. The clear supernatant was separated and amount of untrapped drug was determined by UV spectrophotometer at 226 nm.

The % entrapment was determined by following formula:

Percentage entrapment = Total drug content-Free drug content/ Total drug content \times 100.

In vitro drug release studies

The release of drug was determined by using the treated egg membrane mounted on the one end of open tube, containing ethosomes (equivalent to 10 mg drug). 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 and 12 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} 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 Gliclazide from the prepared ethosomes were analyzed by fitting the release data in to various kinetic models.

Release kinetics

Zero order kinetics

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation

$$Q_t = Q_0 + K_0 t$$

Where,

Q_t = Amount of drug dissolved in time t,

Q_0 = Initial amount of drug in the solution and

K_0 = Zero order release constant.

First order kinetics

To study the first order release rate kinetics the release rate data were fitted to the following equation.

$$\log Q_t = \log Q_0 + K_1 t / 2.303$$

Where,

Q_t = Amount of drug released in time t,

Q_0 = Initial amount of drug in the solution and

K_1 = First order release constant.

Higuchi model

Higuchi developed several theoretical models to study the release of water soluble and low-soluble drugs incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The higuchi equation is

$$Q_t = K_H . t^{1/2}$$

Where, Q_t = Amount of drug released in time t and,

K_H = Higuchi dissolution constant.

Kormeyer - peppas release model

To study this model the release rate data is fitted to the following equation

$$M_t / M_\infty = K . t^n$$

Where, M_t / M_∞ = Fraction of drug release,

K = Release constant,

t = Drug release time and

n = Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form.

Stability studies

To confirm the stability of ethosomal formulation, 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 and 4 ± 2 °C in stability chamber (Thermo lab India). % entrapment, drug particle size and drug release were fixed as physical parameters for stability testing.

Evaluation of Gliclazide ethosomal gel⁸

Physical examination

The physical appearance of color, consistency texture and greasiness these all features were done for ethosomal gel formulation.

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 ethosomal 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} 226 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. ethosomal 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 °C with stirring at 500 rpm throughout the experiment. At fixed time interval, 5ml of aliquots was withdrawn

for every 1, 2, 4, 6, 8, 12 and 24 hrs from receiver compartment through side tube and analyzed by UV spectrophotometer at λ_{max} 226 nm.

In vivo studies

Skin irritation test⁹

Four young Wister albino rats were taken for skin irritation studies. Hair on the back area (approximately 6 cm² 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.

Hypoglycemic activity^{11,12}

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.

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 2 groups (n=4) of diabetes rats. The rats as treated as following.

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

Group II- ethosomal gel contains Gliclazide.

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 ethosomal gel

Intermediate stability testing studies was performed for 6 months as per ICH guidelines. The optimized gel formulation was kept at 30 ± 2 °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

Gliclazide is a white, crystalline, freely soluble in methanol and soluble in chloroform and poor soluble in water. Melting point was determined by capillary method and it was found to be 181°C, which complied with IP standards, thus indicating the purity of drug. FTIR spectra of pure Gliclazide showed sharp characteristic peaks at 1710, 1162, 1089, 997, 920, 667cm⁻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 ethosomes formulation. Comparative study of FTIR graphs are showed in Figure No.1 and 2.

Ethosomes were prepared using film deposition on carrier method using vacuum rotary evaporator. The result showed in Table No.4; we observed that, increase in the concentration of soya lecithin. 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 ethosomes formulation F3 as shown in Figure No.3. The spherical structure in the images confirmed the formation ethosomes that is confirmed the incorporation of lipids and drug. Zeta potential of optimized formulation F3 was found to be - 58.87 mV, which indicates that the formulations are good to be stable.

The release of drug from ethosomes formulation was varied according to concentration of soya lecithin. The progressive increase in the amount of drug diffused through a dialysis membrane from formulations F1 to F4 attributed to gradual increase in soya lecithin. It has been concluded that, if we increase the concentration of soya lecithin, the diffusion of drug also increases. The amount of drug diffused from formulation F3 was showed 86.12 % which was higher among the formulations F1 to F8 and showed in Figure No.4. Stability studies were carried out for the most satisfactory formulation F3 at 30±2 °C and 65±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 F3 showed negligible changes in evaluated parameters that revealed that the formulations are stable on storage.

The optimized formulation F3 was incorporated in 1% carbopol 934 polymers and gel was prepared. The prepared ethosomal gel formulation was evaluated for the following parameters.

Physical appearance

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

pH measurement

The prepared Gliclazide ethosomal gel was checked for their pH and the formulation was found to be 6.09. Therefore there is no need for adjusting pH of the formulation.

Viscosity

The viscosity of gels formulation was determined and formulation F3-G1 showed 12160 cps.

Drug content

The prepared Gliclazide ethosomal gel was subjected to drug content uniformity and it was found to 4.23 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 Gliclazide from the gel formulation is given in Figure No.5. However, the results clearly show that the gels have ability to retain the drug for prolonged periods. The % CDR of ethosomal gel formulation F3-G was found to be 80.37 % 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.

Skin irritation studies

The skin irritation study of ethosomal gel formulations F3-G was performed and tabulated in Table No.5. The Average primary irritation index of formulations F3-G was found to be 0.16, and it shows that the ethosomal 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 Gliclazide ethosomal gel formulation F3-G1 as showed in Table No.6. The pure Gliclazide suspension was administered orally. A maximum reduction in blood glucose level was observed within 2 hrs. The Gliclazide ethosomal gel formulation F3-G1 both

are showed significant reduction in blood glucose level but in sustained manner as compared to oral Gliclazide in PBS pH 7.4. 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 Gliclazide ethosomal gel formulation F3-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 ethosomal gel

Stability studies of ethosomal gel formulation F3-G1 shows negligible changes in pH, drug content and % CDR revealed that the formulations are stable on storage.

Table No.1: Formulation design for the preparation Gliclazide ethosomes

S.No	Formulation code	Drug (mg)	Soya lecithin (%w/v)	Ethanol (%w/v)	Propylene glycol (%w/v)
1	F1	0.5	1	30	10
2	F2	0.5	2	30	10
3	F3	0.5	3	30	10
4	F4	0.5	4	30	10
5	F5	0.5	4	10	10
6	F6	0.5	4	20	10
7	F7	0.5	4	40	10
8	F8	0.5	4	50	10

Table No.2: Formulation design for the preparation Gliclazide ethosomal gel

S.No	Ingredients	F3-G1
1	Gliclazide ethosomes (containing pure drug in mg)	20
2	Carbopol 934(mg)	50
4	Dimethyl sulfoxide(mg)	0.11
5	Methyl paraben(mg)	0.75
6	Distilled water	Up to 5gm

Table No.3: Standards for skin irritation study¹⁰

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

Table No.4: Vesicle size, % Drug content and % Entrapment efficiency of ethosomes formulations

S.No	Formulation code	Average vesicle size in μm	% Drug content	% Entrapment efficiency
1	F1	4.62	90.59	79.53
2	F2	6.13	92.42	81.65
3	F3	6.98	98.95	87.89
4	F4	6.18	95.02	85.43
5	F5	8.68	87.23	65.67
6	F6	7.96	89.95	60.20
7	F7	7.34	92.29	55.80
8	F8	7.02	91.59	53.65

Table No.5: Reading after Skin irritation study of ethosomal gel formulation F3-G1

S.No	Skin responses	Days	Score	
			Rat 1	Rat 2
1	Erythma and Scar formation	1	0	0
		3	0	1
		7	1	0
2	Edema Formation	1	0	0
		3	0	0
		7	0	0
3	Primary irritation index (PII)	--	0.16	0.16

Average Primary irritation index of formulation F3-G =0.16

Table No.6: Hypoglycemic activity of Gliclazide ethosomal gel formulation F3-G

Group-I = Oral, Group-II = F3G1 (Topical)

S.No	Time in hrs	% Reduction in blood glucose level	
		Group-I	Group-II
1	1	50±1.12	21±2.31
2	2	56±1.27	27±2.14
3	3	41±1.92	38±2.59
4	4	38±2.42	45±2.97
5	6	22±1.29	38±2.38
6	8	-	36±1.42
7	12	-	23±1.64
8	24	-	08±1.96

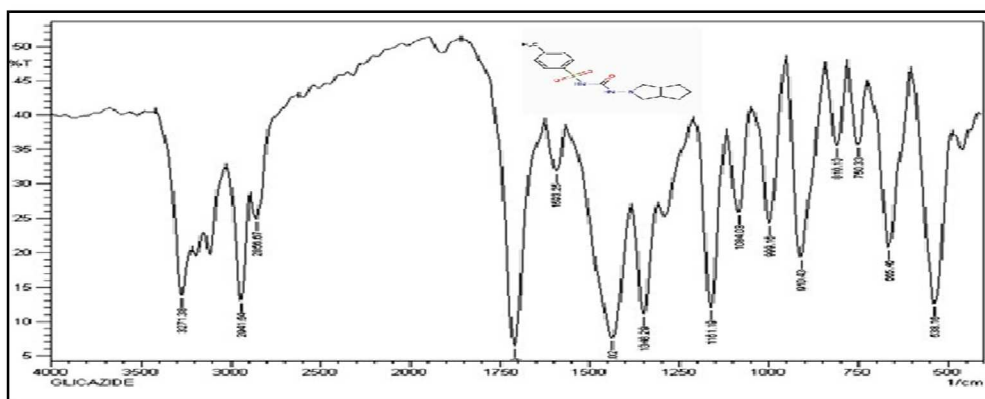


Figure No.1: FT-IR Spectroscopy of Gliclazide

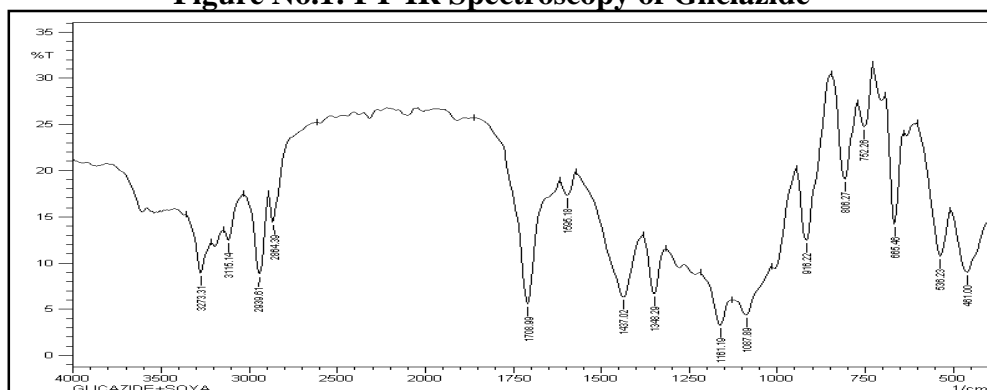


Figure No.2: FT-IR Spectroscopy of Gliclazide + Soya lecithin

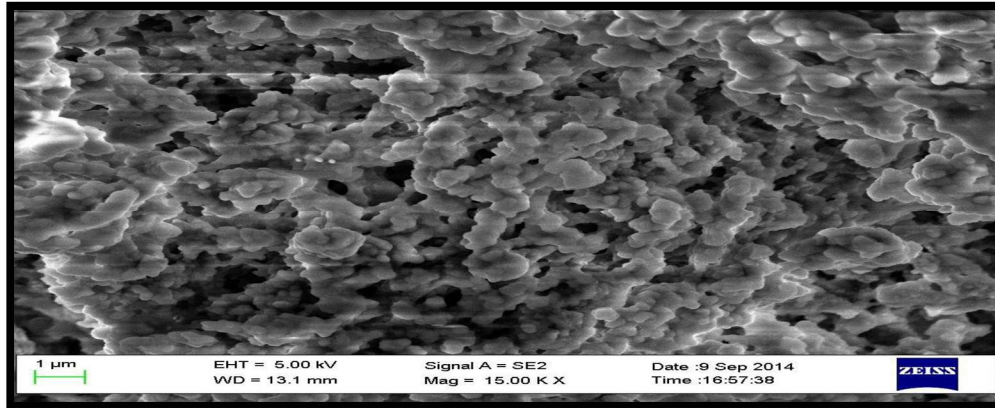


Figure No.3: Scanning electron micrograph of ethosomes formulation F3

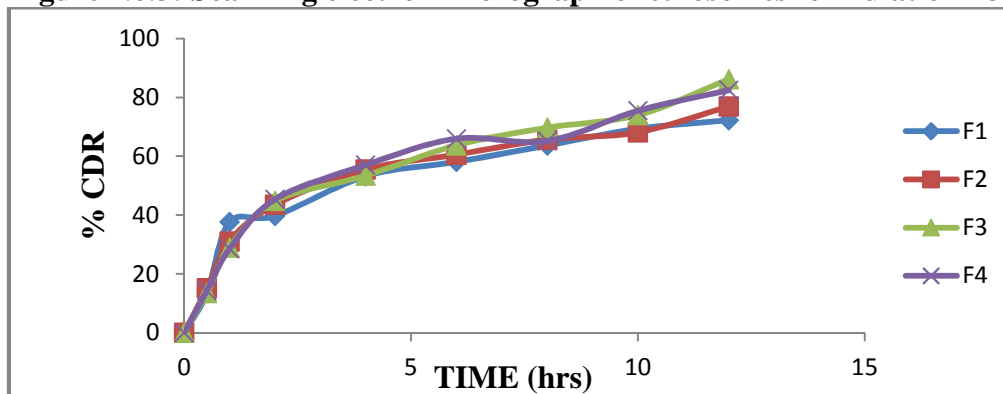


Figure No.4: % CDR of ethosomes formulations

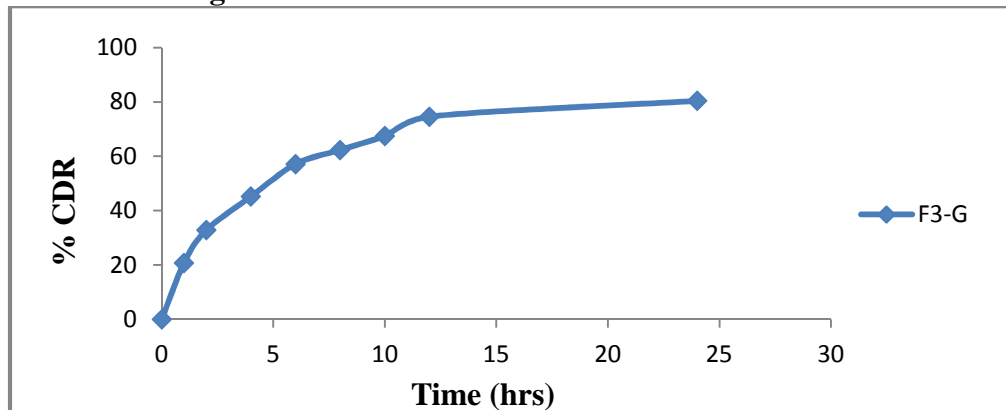


Figure No.5: % CDR of ethosomal gel formulation F3-G

CONCLUSION

Ethosomes were successfully formulated and characterized. Preformulation studies Glucosylated Glucosylated was carried out by determination Melting point, solubility, λ_{max} . The obtained results complied with IP standards, thus indicating the purity of drug. The drug-excipients compatibility by FT-IR confirmed

no significant interaction between drug and selected excipients. F3 was taken as a optimized formulation because of more vesicle size, high drug content, high entrapment efficiency, vesicle are smooth in nature, sufficient stability and better controlled release compare to other formulation and these two was taken for stability studies and preparation of gel.

Stability studies of selected formulation F3 showed that, negligible changes in vesicle size, entrapment efficiency and % CDR revealed that the formulations are stable on storage. The optimized formulation F3 were incorporated in 1% carbopol 934 polymers and gel was prepared. Formulations were evaluated for physical characteristics such as physical nature, pH and drug content estimation pH and Viscosity analysis studies and observed from the result the prepared gel fulfill the requirements for usage. *In vitro* drug release study confirmed that the Gliclazide ethosomal gel formulation has be F3-G better potential of sustaining drug release. Skin irritation study shows that prepared ethosomal gel formulation are compatible with no skin irritation to animal skin and can be used for further studies.

ACKNOWLEDGEMENT

All authors would like to thanks Bharathi College of Pharmacy, Bharathinagara, Mandya, and Karnataka, India for supporting for the fulfillment of this work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Vijayakumar K S. et al. Effect of soyalecithin concentration on formulation of ethosomes, *International Journal of Research in Pharmaceutical and Nano Sciences*, 3(5), 2014, 450-460.