Archives of Pharmaceutical Sciences and Research
Vol 13 Issue 1 114-121 June 2023 Research Article

Development and Evaluation of Metformin Ethosomal Formulation for Diabetes

Kiran R[1], Dr. A Geethalakshmi[1], Suprith D[1]
Department of Pharmaceutics, R.R college of Pharmacy, Bangalore-560090, Karnataka.

ABSTRACT

The main aim was formulation and evaluation of Metformin ethosomes for Diabetes Mellitus. The Metformin Ethosomes was prepared by cold method and evaluated for vesicular size, shape, entrapment efficiency and in – vitro drug release. Total nine formulation (F1 – F9) of ethosomes was prepared with the different concentration of phospholipid (1,2,3% w/w) and ethanol (20,30,40% w/v). Ethosomes were evaluated for vesicular shape, size, entrapment efficiency and in–vitro studies. The (F5) with as the best formulation as it gives the maximum in–vitro release. The stability studies were performed on F5 formulation at two different temperatures of 25±2ºC and 4±2ºC for the period of 3. The formulation incorporated into carbopol 934 (1, 1.5, 2% w/w) as the base. The concentration of carbopol 1.5%w/w gives the maximum in–vitro release of 91.30% in the dialysis membrane and in ex-vivo release of 92.72 in the animal membrane of rat. The results confirmed ethosome as the potential candidate for transdermal delivery of Metformin.

KEYWORDS: TDS, Metformin, Ethosomes, Diabetes Mellitus, Phospholipid, Lecithin.

INTRODUCTION

Ethosomes are nanovesicle consist of phospholipid, cholesterol, Ethanol and water .It enable drugs to reach the deep skin layers of the systemic circulation. They are soft, malleable vesicles known for the Skin lipid bilayer organisation and penetrate into the stratum corneum because of high concentration of ethanol and phospholipids. It is the hydrophilic drug entrapped in aqueous core of the lipid membrane1.

Antidiabttic medicines are used to treat Diabetes. Metformin is a drug us for the Diabets. It is aslo recommn for polycystic ovarian syndrome and gestational diabetes. Metformin is recommended drug for pre-diabetes. Methormin have antiaging, anticancer Propertics and employed for transdermal[2,3,4].

The purpose of this research work was to formulate, in-vitro and ex-vivo evaluation of Metformin thosomes using various polymers such as Phospholipid, Lecithin, Ethanol and cholestrol as polymer by cold to imprive bioavailability and sustain release action of metformin due ti metformin having short half life for 6 hours and neglible protein binding.

MATERIALS AND METHODS

Material

Metformin was collected as gift sample from Ishan Labs Limited, Bangalore. Phospholipid, Ethanol, Cholestrol, Propylene glycol, Triethanolamine and Carbopol 934 from S.D. Fine Chemical. All samples were analytical grade.

Methods

Preformulation Studies

Solubility: The solubility of selected drug was determined in Distilled water, Ethanol, Dimethyl formamide and phosphate buffer pH 7.4.

Melting point: Melting point of metformin was done by Thiels tube by loading the sample in capillary tube and attached thermometer and rubber band.

FTIR: The compatibility study of Metformin, lecithin and cholesterol were perfomed instrument Tensor 27 for the Scanning electron microscopy for vesicle size and shape

Preparation of Ethosomes

Metformin Ethosomes was prepared by cold method. Metformin, lecithin, cholesterol were dissolved in ethanol and the mixture was heated for 300ºC in a closed vessel. Water and propylene glycol was added to mixture and temperature maintned at 300ºC. Then the ethosomes were sonicated and stored at 40ºC.

Preparation of Gel Base

Carbopol was weighed and soaked in a required quantity of distilled water for 3hours, the mixture was transferred to mechanical stirrer at 100rpm to form homogenous viscous solution. The mixture was neutralized by drop wise addition of

triethanolamine. Mixing was continued until a formation of a transparent gel.

Determination of pH

The pH of gels average of 3 samples was taken using a calibrated pH meter.

Rheological Studies

Rheological study was carried out by Brookfield viscometer by selecting suitable spindle and at 3, .6, 1.5, 3, 6, 12, 30, 60rpm. Preparation was kept in 30ml beaker which was set up to spindle groove was dipped avoiding trapping of air bubbles. Start the motor after entering the spindle number. Floating point display is used for the viscosity. Spindle was selected by trial- and-error method.[5]

Spreadability

The two glass slides are taken. At the lower slide sufficient amount of gel was placed and covered by upper slide. Upper slide was pressed by uniform weight of 20g for 2hrs and the time of slide is measured

Turbidity Measurement

The ethosomal solution is hydrated and observed by turbidometry by taking 500 NTU and observed

Drug Entrapment Efficiency

Ethosomal gel was added in phosphate buffer 7.4 and sonicated for 30min and then centrifuged for 10000 rpm for 30min. Then supernatant was diluted with pH 7.4 phosphate buffer and the was assayed by spectrophotometrically using UV – Visible spectrophotometer at 233 nm.[6]

Drug Content Analysis

Ethosomes equivalent to 20mg drug were taken into a standard volumetric flask and were lysed with 25ml of methanol and diluted to 100ml . Then 10ml were take and 100ml with saline phosphate buffer

In-vitro Diffusion Studies

The in-vitro studies were peroformed by taking egg membrane in donor compartment and he receptor compartment will be loaded with buffer

Prepration of Metformin Ethosomal Gel

The best formulation is added to the carbopol with different concentration and triethanolamine is added by drop wise for the formation of transparent gel. [8]

and donor compartment is loaded with ethosomal gel. At (1,2,3,4,5 to 12h) 5ml samples is withdrawn from receptor medium and immediately replaced . Then the samples withdrawn are analyzed spectrophotometrically.

SEM Analysis

A 0.2 gm of the ethosomal gel in a glass cover of the instrument Hitachi 3400 and observed in the 10-100 micrometre.

Ex- Vivo Diffusion Studies

The adult albino rats(10-12weeks) weighing 200-250g are taken and freed from hairs. Then the subcutaneous and fat tissue is removed from abdomen skin and washed in normal saline and dried between two filter papers. The skin is mounted on donor compartment and the receptor compartment will be loaded with buffer and donor compartment is loaded with ethosomal gel. At (1,2,3,4,5 to 12h) 5ml samples is withdrawn from receptor medium and replaced. Then the samples withdrawn are analyzed spectrophotometrically.[9]

Kinetics of drug release

The study of in-vivo were plottes for zoro, first, higuchi and peppas release and by comparing R2 the release kinetics is determined. [10]

Stability studies

Stability studies is according to ICH guidelines at Accelerated Stability Studies were carried on best formulation at 25±20C (60±5%RH), 4±20C (40±5%RH) for the period of 3 months.

RESULTS AND DISCUSSION

Metformin is very slightly Freely soluble in water, soluble in methanol, Phosphate buffer pH -7.4 and Dimethyl Formamide. The melting point of Metformin was found to be 224ºC. Drug-polymer Compatibility studies were carried out by FT-IR Spectroscopy to establish the any possible interaction of excipients with the drug in the formulation. The FT-IR Spectrum of drug alone as well as combination of drug with excipients were obtained and analyzed for

compatibility. FT-IR Spectra of Pure drug showed principal absorption peaks at 347.68 cm-1 (N-H Asymmetric), 3087.74cm-1 (N-H symmetric), 1251.83cm-1 (C-N Strecting), The C-N Strecting, (N-H) Asymmetric and symmetric, C-N Strecting Vibrations were also

Formulation Code	**Drug (mg)**	**Soya lecithin (%w/w)**	**Cholesterol (%w/w)**	**Ethanol (%w/v)**	**Propylene glycol (%w/v)**
F1	50	1	0.005	20	10
F2	50	2	0.005	20	10
F3	50	3	0.005	20	10
F4	50	1	0.005	30	10
F5	50	2	0.005	30	10
F6	50	3	0.005	30	10
F7	50	1	0.005	40	10
F8	50	2	0.005	40	10
F9	50	3	0.005	40	10

Table 1: Formulation of Metformin Ethosomes

INGREDIENTS	FORMULATIONS			
	G1	G2	G3	PD
Ethosomal Suspension (%w/v)	10	10	10	10
Carbopol (%w/w)	1	1.5	2	1.5
Triethanolamine (%w/v)	0.5	0.5	0.5	0.5
Phosphate buffer (%w/v)	q.s	q.s	q.s	q.s

Table 2:Formulation chart for Ethosomal gel

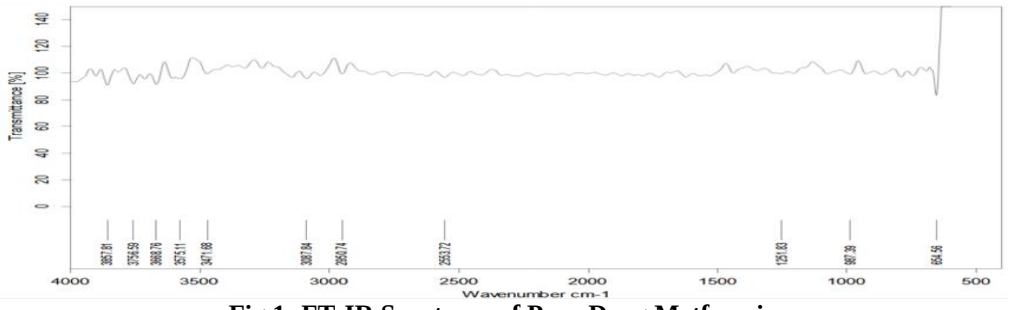


Fig 1: FT-IR Spectrum of Pure Drug Metformin

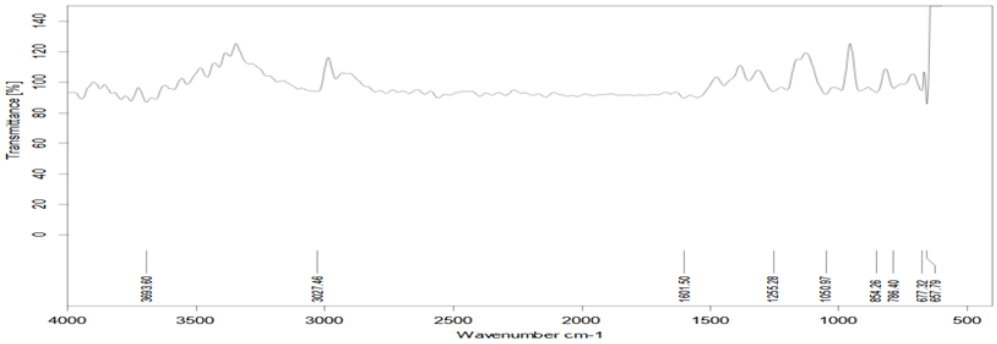


Fig 2: FT-IR Spectrum of Metformin, Soya Lecithin and Cholesterol

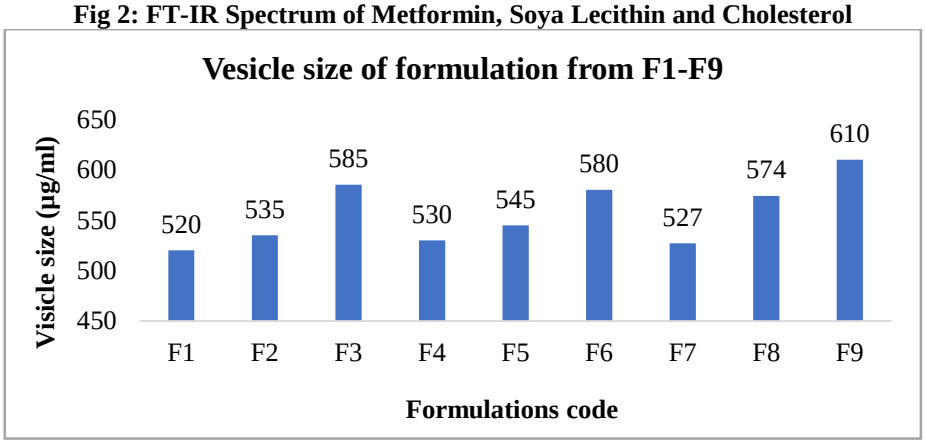


Figure 3: Comparison of vesicle size of ethosomal formulations.

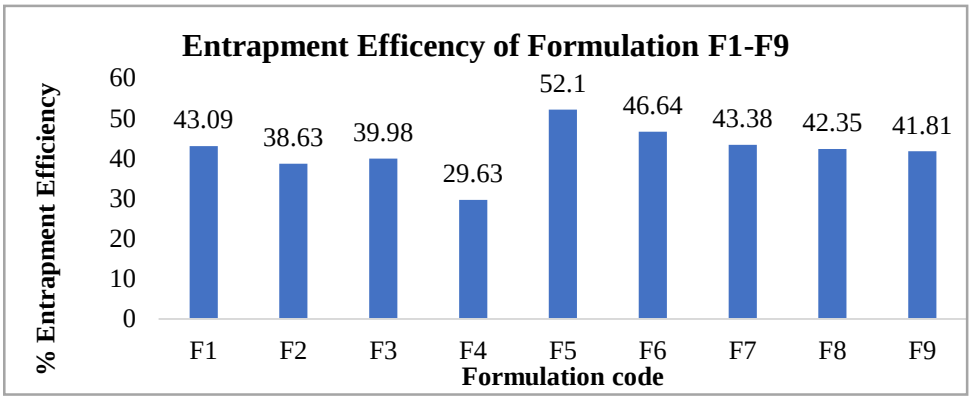


Figure 4: Comparison of entrapment efficiency of Ethosomal formulations.

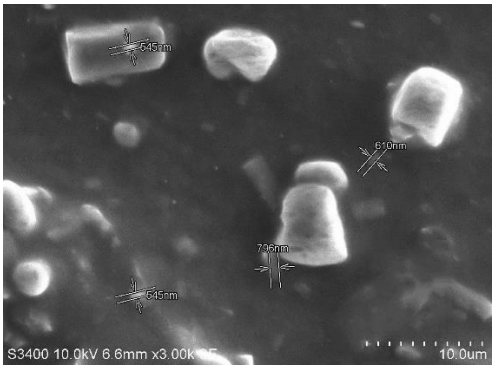
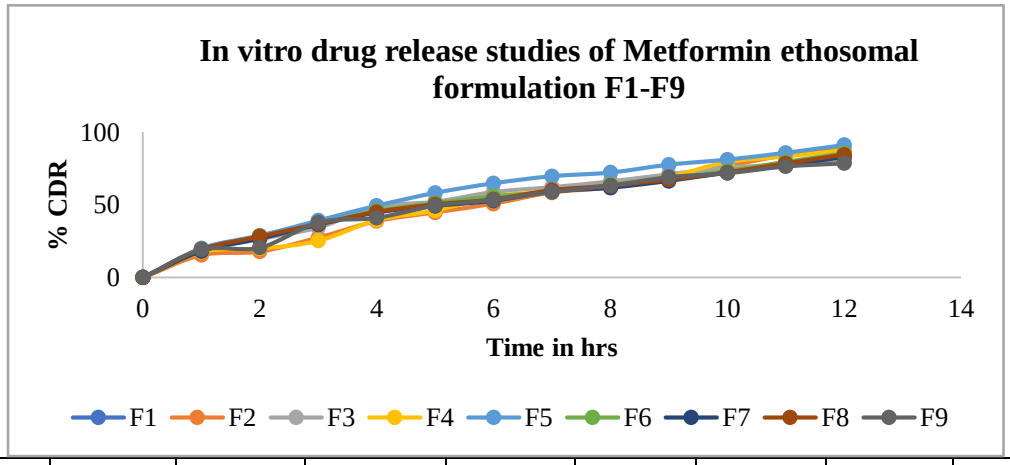


Figure 5: SEM analysis of Ethosomal formulation

Formulation Code	Turbidity (NTU)
F1	200
F2	190
F3	250
F4	246
F5	190
F6	280
F7	190
F8	250
F9	200

Time (hr)	Percentage Cumulative Drug Release								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**



1	19.37	15.44	18.09	17.61	20.10	18.49	18.21	19.37	19.21
2	28.81	17.88	26.50	19.98	28.80	28.39	26.82	28.19	20.55
3	36.93	27.46	34.47	25.31	39.23	36.37	36.44	36.93	37.90
4	45.24	38.81	48.67	39.58	49.48	45.95	44.74	45.12	41.14
5	50.32	44.83	52.20	46.21	58.29	51.13	49.30	50.32	49.47
6	53.75	50.80	58.89	55.76	64.92	55.92	52.84	53.75	53.49
7	59.96	58.56	62.24	59.41	69.78	58.97	59.21	60.12	58.90
8	63.19	61.95	66.20	63.64	72.30	63.92	61.79	63.19	63.32
9	67.39	69.85	71.10	69.42	77.71	68.29	66.46	67.39	69.18
10	72.30	76.74	75.54	79.53	81.21	73.28	72.22	72.30	71.89
11	78.56	84.03	77.84	83.49	85.88	79.67	77.09	78.56	76.50
12	84.51	88.21	79.98	87.75	91.20	85.48	82.95	84.49	78.70

Figure 6: Drug release profile of the Ethosomal formulations F1-F9

Formulation	pH	Spreadability (gcm/sec)	% Drug Content	Viscosity (cps) 12 RPM
G1	7.04	29.27	88.40	10500
G2	7.20	32.66	94.38	11000
G3	6.93	31.35	89.70	11500

Table 7: Evaluation of Ethosomal Gel

Fig 7: Zero order plot of F1- F12 formulation

Table 6: *In-vitro* Release profile of ethosomal formulation F1– F9

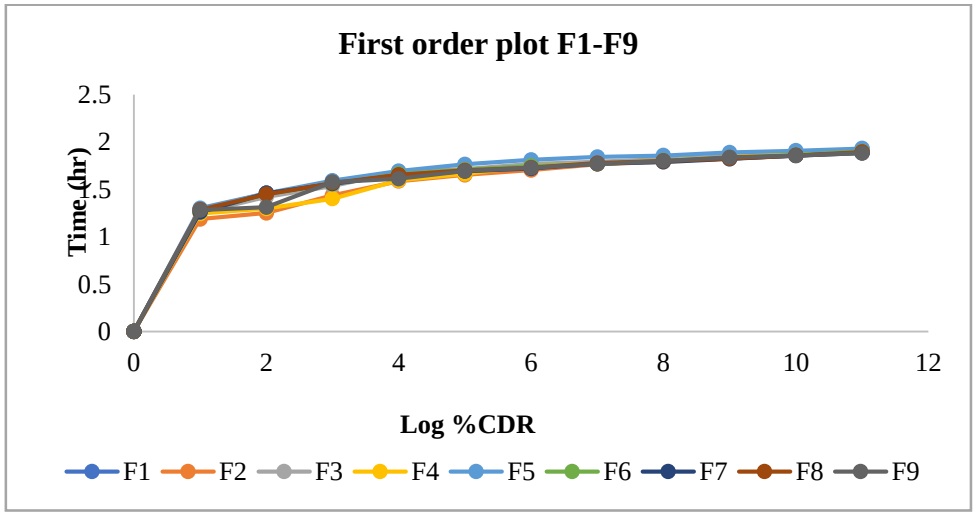
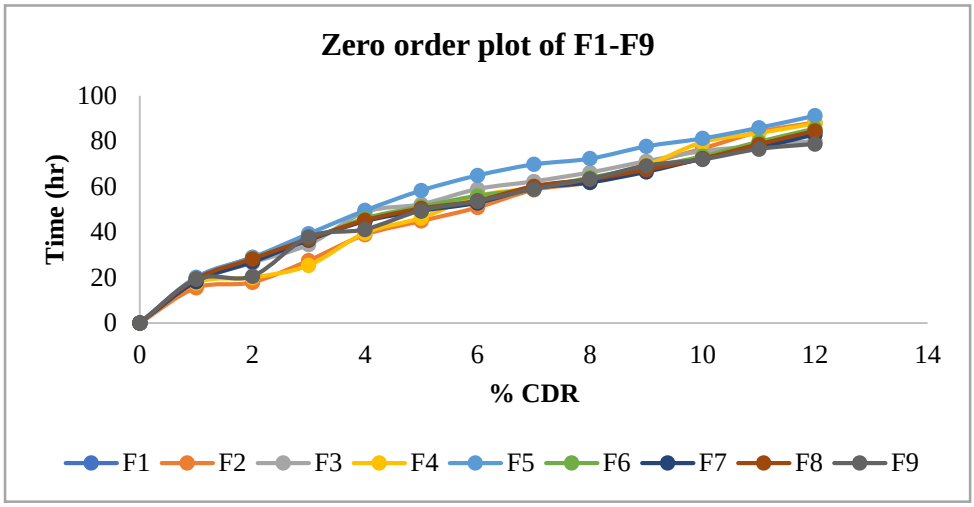


Fig 8: First order plot of F1- F12 formulation



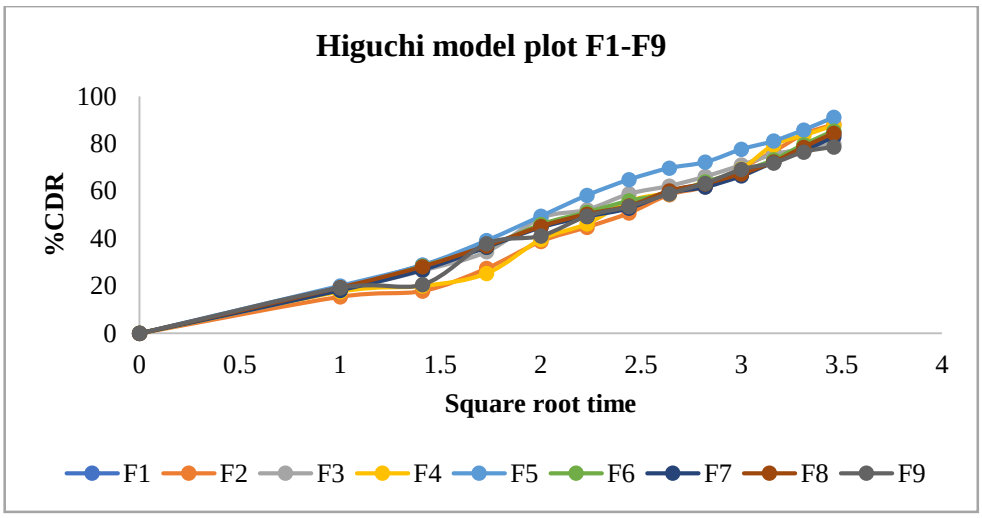


Fig 9: Higuchi's plot of F1- F12 formulation

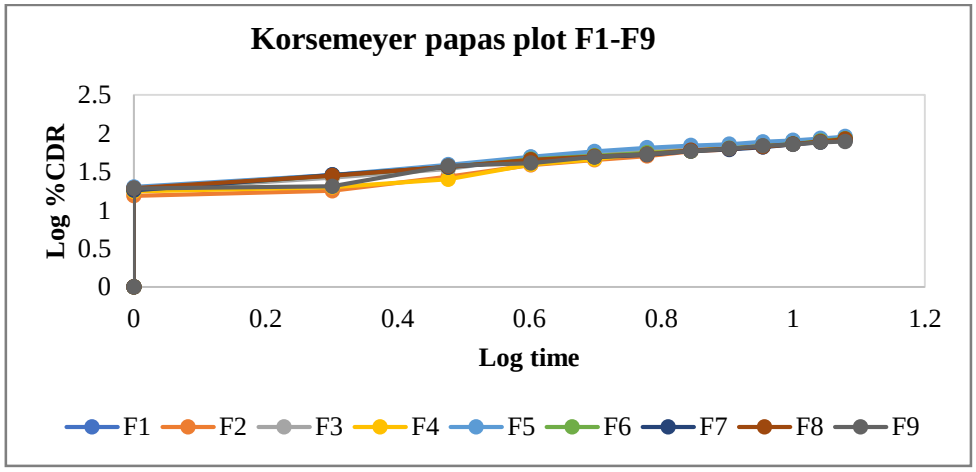


Fig 10: Korsemeyer- Peppas plot of F1- F12 Formulation

noticed in the spectra of physical mixture which contains drug and excipients. The FT-IR Study showed that there is no interaction between drug The vesicle size of Ethosomes were scanned by Scanning Election microscopy and was between520-610 nm. The Entrapment Efficeieny of the ethosomes was between 41.81-52.10 and F5 formulation shows 52.[10] The Scanning and excipients because it shows the characteristic peak of drug and excipients.

In-vitro diffusion studies were carried out for all the prepared formulations from F1 to F12 and the result of diffusion profiles for all the formulations were tabulated in table 6. The in-vitro diffusion profile of Metformin from the transdermal patches containing different concentrations of polymers such as phospholipid, Cholestrol, Ethanol was carried out for 12hrs.

Electon microscopy of ethosomes were round in shape and uniformly dispersed. The Turbidity of all formulation is compared with all formulation by 500 NTU as standard and turbidity of ethosomes is given in table 4.

Among all the formulations, formulation F3 shows minimum drug release 79.98% of the drug in 12hrs and formulations shows maximum drug release 91.20% of the drug in 12hrs. The percentage of cumulative drug release was plotted against Time(hrs) to obtain release profile shown in Figure-6.

Drug content estimation was done and the absorbance was measured by UV Spectrophotometer. Drug content of the developed formulations F5 in G1, G2 and G3 lies in the range of 88.40-% were given in the table 7. Drug content of formulation G3 was found to be higher than other formulations. The pH of Ethosomes was done by digital pH meter. The Formulation F5 in G1, G2 and G3 lies in range 6.93-7.20 were given in table 7. The Spreadibility of the formulation F5 in G1, G2 and G3 lies in range 88.40-94.38. The G2 was found to be higher and were given in table 7. The Rheological stydy was done by Brookfield viscometer by using LV 64 spindle of the formulation F5 in G1, G2 and G3 lies in range 10500-11500 cps at 11 RPM were given in table 7.

In case of formulation F5 applied to ex-vivo skin showed drug release of 91.27% over the period of 12hr. The F5 formulation was found that kinetics release was best explained by the Higuchi and best fitted into Korsmeyer- Peppas model suggesting that the drug was released by Super Case 2 Transport.

Stability studies were carried out on the best formulation as per ICH guidelines Q1C. Stability data of selected F5 formulation stored at STABILITY STUDIES Stability data of selected F5 formulation stored at 25±20C (60±5%RH), 4±20C (75±5%RH) for the period of 3 months. The F5 formulation shows no signifance change in the stability study.

CONCLUSION

The Ethosomes with Metformin, Phospholipid, Cholestrol and ethanol with F5 2% soya lecithin and 30% ethanol was selected as a best formulation among all formulations, due to maximum encapsulation efficiency of 52.10% and drug release of 91.20%. Stability studies were carried out on formulation F5 for three months and no significance change was observed and shows the subtain release action.

REFERENCES

1. Divya A, Ujjwal N, Ethosomes: A review. Int J Pharm Med Res. 2010; 2(2):448- 452.
2. Ziquan Lv, Yajie Guo1. Metformin and Its Benefits For Various Disease. Front Endocrinol. 2020;11-191.
3. https://lakeviewpharmacy.com/articles/topical-metformin-a-solution-for-medicinal-side-effects.
4. https://www.pavilioncompounding.com/transdermal-metformin-therapy-tmt-avoid-unwanted-side-effects.
5. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes- novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. A review. J Cont Rel. 2000;65(3):403-418.
6. Aute PP, Kamble SM, Chaudhari DP, Bhoshale VA,Ethosomes: A comprehensive review. Int J Res Dev in Pharm Life Sci 2012;2(1):218-224.
7. Verma P, Pathak K, Ethosomes: A Review. J Adv Pharma Tech Res 2010;1(3):274-281.
8. Xiao N, Danping Z, Qiong B. Mechanism investigation of ethosomes transdermal permiation. Int J Pharm. 2019;100027.
9. Anjali S, Priyanka R, Meenakshi S, and Satish N. Comparative studies on skin permeation of miconazole using different novel carriers. Int J Pharm Sci Res 2010; 1 (9):61-6.
10. Korsmeyer RW, Peppas NA. Macromolecular and modeling aspects of swelling-controlled systems. Controlled release delivery systems. In: Mansdrofsz, rosemann TJ, ad, Controlled Release Delivery systems. New York. Marcel Dekker;1983(77-90):83