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Research Paper

FORMULATION AND *IN-VITRO* EVALUATION OF LIPOSOMAL DRUG DELIVERY SYSTEM OF METFORMIN HCl

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Metformin is widely used for the treatment of diabetes; the intention of the present study was to formulate Metformin HCl liposomes for a sustained drug delivery system. It have the advantages of dose reduction, less dosing frequency, minimize the side effect, prolong the action of drug and thus achieve better patient compliance. The liposomes were prepared by physical dispersion and ether injection method. Soya lecithin and cholesterol were used for encapsulating the drug, it facilitates to release the medicaments in sustained manner. Chloroform, ether and methanol were used as a solvent. Phosphate buffer pH 6.8 was used as a hydration medium for loading the drug. The final liposome was evaluated in various quality parameters of drug entrapment efficiency, morphological analysis, particle size analysis, *in-vitro* drug release studies and stability studies. In the two methods of metformin liposome formulation the ether injection method showed prolonged action when compared to physical dispersion method. In the parameters of drug entrapment and stability physical dispersion method was shows better results.

Key words: Physical dispersion, ether injection, soya lecithin, cholesterol, morphological analysis, metformin.

INTRODUCTION

Novel Drug Delivery system (NDDS) is a combination of advance technique of dosage form which are far better than conventional dosage form[1].The goal of NDDS is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration[2]. NDDS is combining polymer science, pharmaceutics and molecular biology[3].

Liposomes are colloidal, vesicular structure composed of one or more bilayers surrounding an equal number of aqueous compartment[4]. Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural nontoxic phospholipids. Due to their size and hydrophobic and hydrophilic character (besides biocompatibility), liposomes are promising systems for drug delivery[5]. The sphere like shell encapsulated a liquid interior which contain substances such as peptides, protein,

hormones, enzymes, antibiotics, anti-fungal and anti-cancer agents[4]. Liposome properties differ significantly with lipid composition, surface charge, size, and the method of preparation. Moreover, the choice of bilayer components determines the 'rigidity' or 'fluidity' and the charge of the bilayer. For instance, unsaturated phosphatidylcholine species from natural sources (egg or soybean phosphatidylcholine) give much more permeable and less stable bilayers, whereas the saturated phospholipids with long acyl chains (for example, dipalmitoylphosphatidyl choline) form a rigid, rather impermeable bilayer structure[5].

It has been exhibited that phospholipids impulsively form closed structures when they are hydrated in aqueous solutions. Such vesicles which have one or more phospholipid bilayer membranes can transport aqueous or lipid drugs, depending on the nature of those drugs. Because lipids are amphipathic (both hydrophobic and hydrophilic) in aqueous media, their thermodynamic phase properties and self-assembling characteristics influence entropically focused confiscation of their hydrophobic sections into spherical bilayers. Those layers are referred to as lamellae[6]. Liposomes particle sizes ranges from 30 nm to several micrometers. They consist of one or more lipid bilayers surrounding aqueous units, where the polar head groups are oriented in the pathway of the interior and exterior aqueous phases[7].

MATERIALS AND METHODS

Metformin Hcl, Cholestral and Sodium hydroxide was obtained from Reachem laboratory chemicals, Chennai.

Chloroform and ether obtained from Rankem laboraties, Haryana. Soya lecithin received from the urban platter food co., Mumbai. Potassium di hydrogen phosphate purchased from Merck specialities pvt. Ltd, Mumbai. All other chemicals used were of analytical grade and were used without further purification.

Methodology

Preparation of standard curve of Metformin HCl using pH 6.8 phosphate buffer[8]:

Accurately weighed 100 mg metformin HCl was dissolved in water and the volume was make up to 100 ml using distilled water in a volumetric flask to obtain a solution of 1000 µg/ml. From the above solution 10 ml was pipetted out into a 100 ml volumetric flask and made up to 100 ml using phosphate buffer pH 6.8 to get a stock solution of 100 µg/ml. From this stock solution, aliquots of 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml, 1.2ml, 1.4ml, 1.6ml, 1.8 ml and 2.0ml were pipetted out into a series of 10 ml volumetric flask and made up to mark with phosphate buffer pH 6.8 to get a concentration in the range of 2 to 20 µg/ml. The absorbance

of the resulting solution was then measured at 233 nm using UV Double beam spectrophotometer against phosphate buffer pH 6.8 as blank. The standard curve was obtained by plotting concentration (µg/ml) values in X- axis and absorbance values in Y – axis.

Preformulation studies

The objective of preformulation testing is to generate information useful to the formulation in developing stable and bioavailable dosage forms. The use of preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacious and stable product[9].

a) Solubility

Solubility of Metformin Hcl in water, methanol, phosphate buffer pH 6.8 was determined at room temperature with the help of magnetic stirrer.

b) Melting Point

Melting point determination was done by using melting point apparatus. Small amount of pure drug of Metformin HCl was taken in a capillary tube and it was kept in the melting point apparatus and the melting point was noted[10].

c) Compatibility (Drug – excipients interaction) studies:

FT-IR spectra were taken for the dried samples using FT-IR 8400S (Shimadzu, Japan) to determine the possible interactions between the drug and polymers. The plain drug, lecithin, cholesterol and combination of drug with cholesterol and lecithin in three different ratios (1:1, 1:2 and 1:3) were taken and mixed with KBr.

The samples were compressed to form a pellet using a hydraulic press. The prepared pellets were transformed into

disk. The disk was applied to the centre of the sample holding device and scanned from 4,500 to 400 cm-1 using FT-IR spectrophotometer[11].

Formulation of liposomes loaded with Metformin hydrochloride:

The formulation of liposomes loaded with Metformin HCl was prepared by two different techniques namely, physical dispersion method and ether injection method. In both the techniques ratio of cholesterol was kept as same and the lecithin concentration was increased as 1:1, 1:2 and 1:3.

Physical dispersion method:

Liposomes were prepared by physical dispersion method using different ratio of soya lecithin and cholesterol was kept as constant. In this method the soya lecithin and cholesterol were dissolved in chloroform. Then it was spread over flat bottom conical flask and allowed to evaporate at room temperature for overnight without disturbing the solution for a formation of lipid film. The drug was dissolved in phosphate buffer pH 6.8. It act as an aqueous medium. Then the aqueous medium

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was added to the lipid film for hydration. For this the flask was inclined to one side and aqueous medium was introduced down the side of flask and flask was slowly returned to upright orientation. Then the conical flask was kept on water bath and the temperature was maintained at 37± 2ºC for 2 hours for the completion of hydration. The conical flask was gently shaken until the lipid layer was removed from wall of conical flask and formation a liposomes suspension. Then the formed liposomes suspension was stored at 4ºC for one day for the maturation of liposomes. The prepared liposome suspension was centrifuged at 15,000 rpm for 20 mins. Then the precipitate was collected and diluted with distilled water for further studies[12]. Different batches of liposomes were prepared as per the general method described above and composition for the preparation of liposomes is given in **Table No. 1.**

Liposomes were prepared by ether injection method using different ratio of soya lecithin and cholesterol was kept as constant. In this method the cholesterol and soya lecithin were dissolved in ether and methanol. The drug was dissolved in phosphate buffer pH 6.8. It act as an aqueous medium. The aqueous medium was heated to 60°C. The method involves injecting drop by drop of ether-lipid solutions into the above warmed aqueous medium. The ether vaporizes upon contacting the aqueous phase, and the dispersed lipid forms primarily unilamellar liposomes. Then the product was collected and it was stored at 4°C for maturation of liposome. Then prepared liposomal suspension was centrifuged at 15,000 rpm for 20 mins. The precipitate was diluted with distilled water for evaluation studies[13]. Different batches of liposomes were prepared as per the general method described above and composition for the preparation of liposomes is given in **Table No. 1**.

Table 1: Formulation of Metformin HCl liposomes

S. No.	Ingredients	Physical dispersion method			Ether injection method		
		F 1	F 2	F 3	F 4	F 5	F 6
1.	Cholesterol	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
2.	Lecithin	100 mg	200 mg	300 mg	100 mg	200 mg	300 mg
3.	Metformin HCl	10 gm	10 gm	10 gm	10 gm	10 gm	10 gm
4.	Ether	-	-	-	7 ml	7 ml	7 ml
5.	Methanol	-	-	-	3 ml	3 ml	3 ml
6	Chloroform	5 ml	5 ml	5 ml	-	-	-
7.	Phosphate buffer pH 6.8	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

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Evaluation of liposomes:

Determination of percentage drug entrapment efficiency:

Drug entrapment efficiency was calculated by using centrifugation method. 10 ml of liposome suspension was taken and centrifuged at 15,000 rpm for 20 mins. The supernatant liquid was collected and suitably diluted. Then the absorbance was taken at 233 nm with the help of UV double beam spectrophotometer using pH 6.8 as a blank. The drug entrapment efficiency was calculated from the following formula[14].

Metformin HCl content at 233 nm with pH 6.8 as blank using double beam UV double beam spectrophotometer[16].

Particle size determination:

The particle size determination is done by using Shimadzu SALD – 2300 (WingSALD II: Version 3.1.1). Groups of particles are dispersed in a liquid medium and measured as they are circulated between the flow cell, which is placed in the measurement unit, and a dispersion bath in the sampler. The dispersion bath incorporates a stirrer and an ultrasonic sonicator. A pump delivers the dispersed suspension to the flow cell. The pump is specially designed to ensure both liquid medium and the particles are circulated. It can be controlled from a computer. Organic solvents can be used as dispersion media[17].

Stability studies:

The prepared Metformin HCl liposomes for all the formulations were viewed under for observing the vesicle formation and discreteness of dispersed vesicles. A slide was prepared by placing a drop of liposome dispersion on a glass slide and cover slip was placed over it and this slide was viewed under optical microscope at 40X magnification. Photographs were taken to prepared slides using digital camera[15].

Morphology analysis:

$$\text{Total entrapment efficiency} = \frac{\text{Amount of drug in supernatant liquidl}}{\text{Total Amount of drug}} \times 100$$

***In vitro* drug release study:**

The *in vitro* release for all the formulated Metformin HCl liposomes were carried out for 8 hours in phosphate buffer pH 6.8. The studies were carried in USP dissolution apparatus II (Paddle) at 37ºC ± 0.5ºC and 50 rpm speed. 900 ml of phosphate buffer pH 6.8 was used as a dissolution medium. Equivalent to 100 mg of Metformin HCl liposome was taken in a dissolution jar contains dissolution medium and the paddle was rotated at 50 rpm. 1 ml of samples were withdrawn at every 30 minutes up to 480 minutes and make up the sample to 10 ml with pH 6.8 buffer and analyzed for The behavior of the liposome to retain the drug was studied by storing the liposome at two different

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temperature conditions, i.e., 4ºC (refrigerator RF), 25°C±2ºC for a period of 1 month. The liposomal preparations were kept in sealed vials. At 30th day the samples were analyzed for the drug content following the same method described in % drug encapsulation efficiency and *in vitro* drug release. And also the liposomes were studied for their morphology[18].

RESULTS AND DISCUSSION

Calibration of Metformin Hcl using phosphate buffer pH 6.8:

The Standard Calibration curves of metformin hydrochloride were prepared by using phosphate buffer pH 6.8 and absorbance were analyzed in 233nm. The correlation coefficient was found to be 0.9994. The results indicate Metformin hydrochloride obeys the beer's law within the concentration range of (2-20µg/ml). Calibration plot of metformin was shown in **Table No. 2** and **Figure 1.**

Preformulation studies

a) **Solubility**The solubility of raw drug was determined by dissolving in distilled water, methanol and phosphate buffer pH 6.8. The drug was found to be freely soluble in water, soluble in methanol and phosphate buffer p H 6.8.

b) **Melting point**

It was found to be 224ºC which was within the specification range of standard. So it confirmed Metformin HCl present in raw material of drug.

c) **Compatibility (Drug – excipients) studies**

The FT – IR studies of pure Metformin HCl, cholesterol, soya lecithin and combination of Metformin HCl, cholesterol and soya lecithin were conduct to study the interaction between the drug and excipients. FT- IR spectral analysis showed that the fundamental peaks and patterns of the spectra were similar both in pure drug and combination containing drug and highest proportion of excipients. This indicated that there was no chemical

Table 2: Calibration curve of Metformin

Sl. No.	Concentration (µg/ml)	Absorbance at 233 nm
	2	0.189
	4	0.370
	6	0.524
-	8	0.699
	10	0.858
	12	1.055
	14	1.244
	16	1.394
	18	1.568
	20	1.716

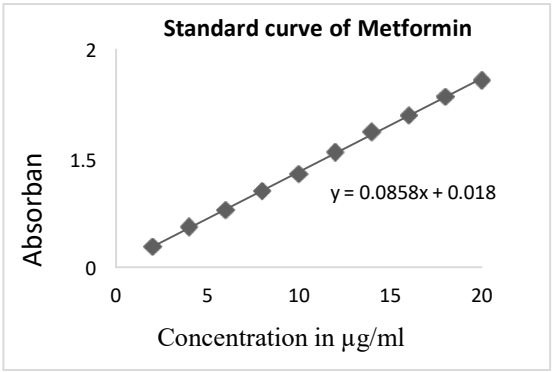

Figure 1: Calibration curve of Metformin

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interaction between Metformin HCl and the other excipients used in the formulations. The spectral data's are presented in **Table 3-6** and spectral peaks were presented graphically in **Figure 2-5.**

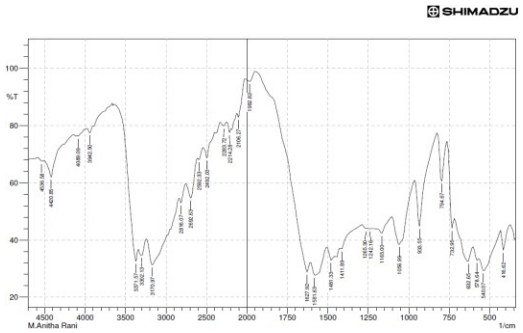

Figure 2: FT – IR of pure Metformin

Table 3: FT – IR of pure Metformin

Wave length (cm^{-1})	Functional group
3372	N-H stretching
1582	Amino N-H bending
1466	CH3 bending alkanes
1057	C-N Stretching
957	Alkene C-H bending

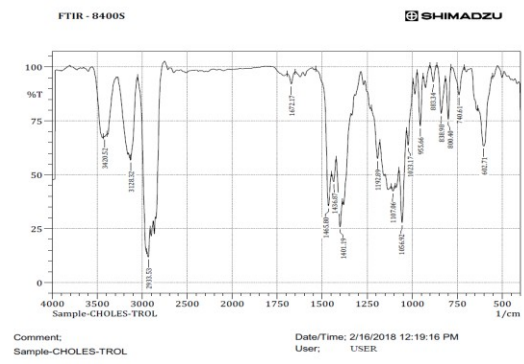

Figure 3: FT – IR of cholesterol

Table 4: FT – IR of cholesterol

Wave length(cm^{-1})	Functional group
3421	N-H stretching
1466	CH3 bending alkanes
1057	C-N Stretching
955	Alkene C-H bending

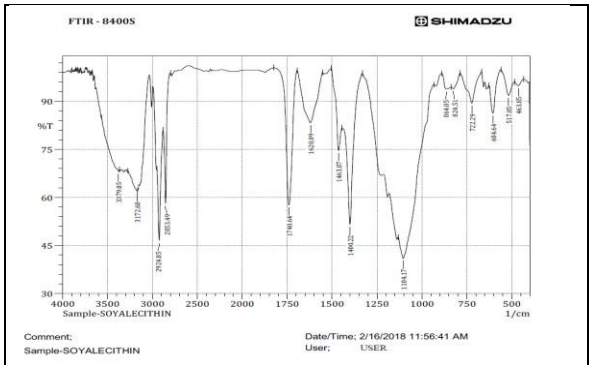

Fig 4: FT – IR of lecithin

Table 5: FT – IR of lecithin

Wave length (cm^{-1})	Functional group
3372	N-H stretching
1582	Amino N-H bending
1466	CH3 bending alkanes
1057	C-N Stretching
957	Alkene C-H bending

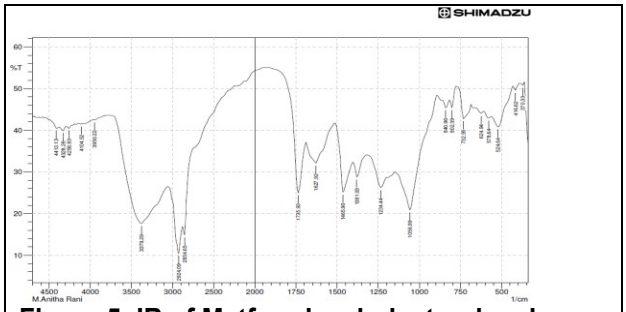

Figure 5: IR of Metformin, cholesterol and lecithin

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Table 6: FT – IR of combination of Metformin, cholesterol and lecithin.

Wave length (cm^{-1})	Functional group
3372	N-H stretching
1582	Amino N-H bending
1466	CH3 bending alkanes
1057	C-N Stretching
957	Alkene C-H bending

Table 7: Particle size range

Sl. No.	Formulations	Particle size range
1	F 1	30.617 – 1.563 µm
2.	F 2	19.023 – 1.563 µm
3.	F 3	0.071 -0.031µm
4.	F 4	24.133 – 1.563 µm
5.	F 5	0.081 – 0.031 µm
6.	F 6	0.071 -0.031µm

respectively and formulations F 4, F 5 and F 6 were 30.47%, 39.58% and 39.69% respectively. The results may specify physical dispersion method have better drug entrapment efficiency than ether injection method.

Morphology analysis

The morphology characters of liposomes were analyzed by optical microscopy (Olympus Opto System, India) and the images were taken using digital camera. The formulation F 1 F 6 microscopic images were showed in **Figure No. 6.**

Particle size analysis

The particle size analysis was carried out by malven particle size analyzer for all the prepared liposome formulations. The particle size for all the formulated liposomes were

Formulation of Metormin HCl liposomes.The Metformin liposome was prepared by physical dispersion method and ether injection method using Soya lecithin and cholesterol in different ratios as per formula designed in Table 1. The F1 to F3 formulation prepared by physical dispersion method, F4 to F6 formulation prepared by ether injection method. **Evaluaton of Metormin HCl Liposomes Percentage drug entrapment efficie** found be in the range of 30.617 µm to 0.031µm as shown in **Table No.7.** The particle size data showed that when the concentration of soya lecithin was increased the particle size was decreased invariably the Metformin HCl liposomes in prepared by both methods. The particle size of Metformin HCl liposomes of F 3 and F 6 were found to be lower when compared with other formulations this may be due to higher concentration of soya lecithin.

***In vitro* drug release studies**

The cumulative percentage drug release of formulations F 1, F 2 and F 3 were found to be 103.03±2.47, 91.92±2.72 and 82.12±2.51 respectively in 8 hours. The formulation F 1 shows faster release than formulations F 2 and F 3 due to the lower concentration of soya lecithin. The cumulative percentage drug release of formulations F 4 was found to be 100.58 ± 1.58 at the end of 7 hours. And the

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cumulative percentage drug release of formulations F 5 and F 6 were found to 85.06±1.73 and 81.39±1.12 respectively in 8 hours. The formulation F 4 show faster release than formulations F5 and F 6. While the concentration of soya lecithin was increasing it decrease the release of drug.

The prepared liposomes F 1 to F 6 showed sustained release of drug. When increased ratios of soya lecithin also sustain the release of drug was increased in both methods of preparations. The **Figure No. 12 and 13** shows the formulation F 1, F 2 and F 3 and F 4, F 5 and F 6 respectively in 8 hours.

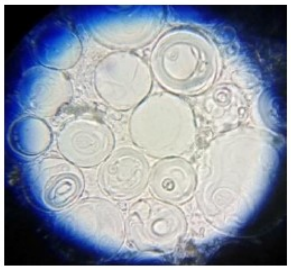

Figure 6: Microscopic image (45 X) of F 1 formulation

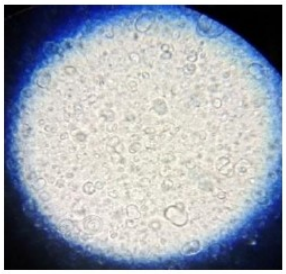

Figure 7: Microscopic image (45 X) of F 2 formulation

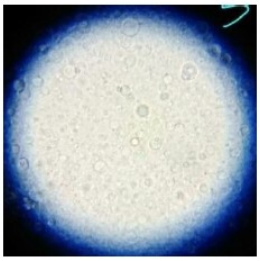

Figure 8: Microscopic image (45x) of F 3 formulation

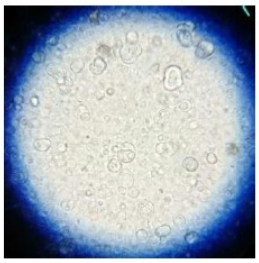

Figure 9: Microscopic image(45x) of F 4 formulation

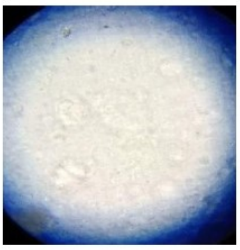

Figure 10: Microscopic image (45x) of F 5 formulation

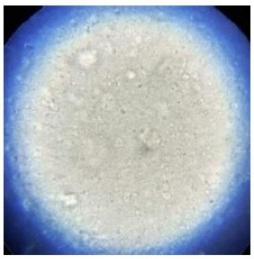

Figure 11: Microscopic image (45x) of F 6 formulation

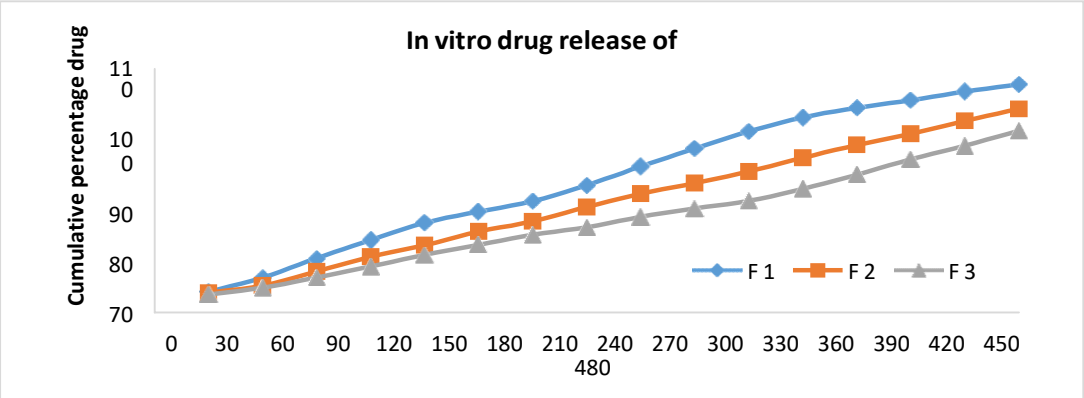

Figure 12: Comparative cumulative percentage drug release of Metformin HCl liposome formulations of F 1, F 2 and F 3

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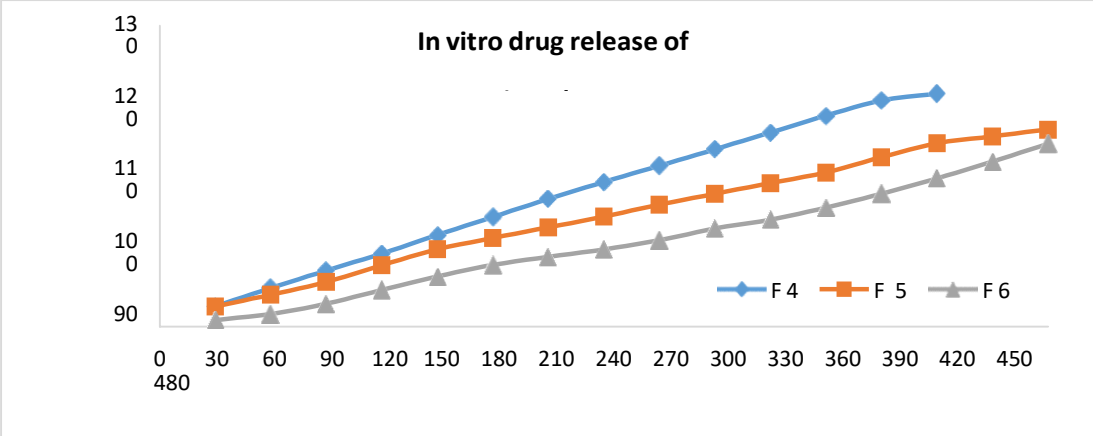

Figure 13: Comparative cumulative percentage drug release of Metformin HCl liposome formulations of F 4, F 5 and F 6

Stability Studies

All the formulations of Metformin HCl liposomes were relatively stable at 4ºC storage condition. The drug leakage percent amounts of original entrapped in liposomes were very small and the amount retained in vesicle had no significant difference after one month as compared to the amount immediately after preparation. But at the storage condition of 25°C±2°C, all the formulations of Metformin HCl liposomes were unstable.

Table 8: Stability study of percentage drug entrapment of Metformin HCl liposomes compared with percentage drugentrapment of immediately after preparation.

Sl. No.	Formulations code	Immediately after preparation (%)	After one month	
			At 4°C	At 25°C±2ºC
1.	F 1	86.60	85.92%	76.87%
2.	F 2	79.90 %	77.99%	70.98%
3.	F 3	73.10 %	72.08%	66.89%
4.	F 4	30.47%	29.35%	24.89%
5.	F 5	39.58%	38.44%	35.39%
6.	F 6	39.69%	38.36%	36.69%

Table 9: *In vitro* drug release of Metformin liposome formulations after stability study, compared with before stability

Sl. No.	Formulation code	Immediately after preparation	After stability study	
			At 4°C	At 25ºC±2ºC
	F 1	103.03±2.47	91.81	73.38
	F 2	91.92±2.72	86.77	68.26
	F 3	82.12±2.51	77.91	64.37
	F 4	100.58±1.12	91.74	87.41
	F 5	85.06±1.73	78.81	61.81
	F 6	79.05±1.03	73.98	63.32

In addition, the result of drug entrapment studies showed higher leakage at higher temperature. This may be due the higher fluidity of lipid bilayer at higher temperature, resulting into higher drug leakage. The drug entrapment results were shown in table no. 8.The morphological characters of Metformin HCl liposomes for **F 1 – F 4** didn't show any characteristic changes after it was stored at 4ºC and 25ºC±2ºC for a period of one month. F 5 and F 6 formulations were showed slightly reduced in the size after it was stored at 25ºC±2ºC for a period of one month but there was no changes for the same formulation when it was stored at 4ºC. Microscopic images of all the formulations (F 1 – F 6) of Metformin HCl liposomes were compared with before and after stability studies. The results show there no significant changes.

After one month, Metformin HCl liposomes formulations F 1 to F 6 were showed difference in *in vitro* drug release profile. Dissolution rate was decreased in all Metformin HCl liposomes formulations at both storage conditions like 4°C and 25ºC±2ºC. The results of *in vitro* drug release of all the formulations at both storage conditions were compared with before and after stability studies and the results were shown in **Table No. 9.**

At storage condition 4°C showed better stability than another condition. This may due to their elevated temperature reduce the stability. But in both storage conditions higher proportion of soya lecithin contains formulations like F 3 and F 6 showed better stability than other their formulations.

SUMMARY AND CONCLUSION

is study concluded that Metformin HCl was successfully prepared as a liposomal drug delivery system by using two different techniques such as physical dispersion method and ether injection method. The liposomes prepared by physical dispersion method showed better percentage drug entrapment when compared with ether injection method. The morphological characters of prepared liposomes were determined with the help of optical microscope. The results of the particle size showed, when the concentration of soya lecithin was increased the size of the particle was reduced. The *in vitro* release showed that as the concentration of soya lecithin was increased the release rate of drug was retarded. Among the two methods ether injection method showed prolonged action when compared to physical dispersion method. The stability studies for all the formulations were performed by keeping the formulations at two different temperatures 4ºC±2ºC and 25ºC±2ºC for a period of 30 days. After the stability period the formulations were tested for morphological analysis, percentage drug entrapment and *in*

vitro drug release and compared with before stability study. There was no change in morphological characters at 4ºC±2ºC, but there was a slight reduced in particles size at 25ºC±2ºC. The percentage drug entrapment was reduced in all the formulations at both the conditions. The *in vitro* drug release was reduced for all the formulations. Liposomes prepared by physical dispersion method showed better stability compared with ether injection method.

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Conflict of Interest

The authors declare that they have no conflict of interest