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Original Research Articles

FORMULATION AND EVALUATION OF LIPOSOMAL DRUG DELIVERY SYSTEM OF METFORMIN HYDROCHLORIDE

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ABSTRACT

The present investigation deals with liposomes. Liposomes are effective in extending the duration of action of drug. In the present study liposomal formulations containing Metformin hydrochloride were prepared and studied. Because of its shorter plasma half-life ($t_{1/2}=1.5$ h) frequent administration by the patient is necessary to maintain steady state plasma concentration. Designing of controlled release formulation is necessary to maintain steady state plasma concentration for longer period of time and to reduce the frequency of administration. So, an attempt was made to formulate liposome of Metformin hydrochloride to sustain its action. Liposome were prepared using film hydration technique using varying concentrations of Soya lecithin and cholesterol and optimize the ideal combination for required drug release. Liposomal formulations were evaluated for Partical size, Percentage yeild, % entrapment efficiency, SEM, FTIR, DSC, In vitro drug release and Accelerated stability studies. The prepared liposomes were rigid, intact and fulfilled all official requirements. The results of in vitro drug release studies showed that release from liposomal formulation was slow and sustained for 7 hrs period. The formulations followed zero order kinetics and release mechanism was non-fickian diffusion from all the formulations. Thus the liposome of Metformin hydrochloride was successfully developed.

Keywords: Liposomes, Thin film hydration, Phospholipid, Duration of action, Metformin HCl.

INTRODUCTION

Metformin HCl is the third generation biguanide drug, which lowers the blood glucose level in the healthy subjects as well as in patients with type 2 diabetes. Metformin HCL belongs to biopharmaceutical classification - III with drug pKa: 12.33 showing small intestine as the major absorption site. Due to its low biological half life (1.5hour), it requires frequent administration. To reduce the dosing frequency and to improve patient compliance prolonged release dosage forms are required. Hence, there is a scope for continued interest and need for developing controlled release formulations.

Novel system of drug delivery offers a means of improving the therapeutic effectiveness of incorporated drugs by providing sustained, controlled delivery and targeting the drug to with drugs into dosage form with the aim of sustaining drug levels and hence drug action is obtained for an extended period of time.

Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. Liposomes are microparticulate lipoidal vesicles which are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. Due to new developments in liposome technology, several liposome-based drug formulations are currently in clinical trial, and recently some of them have been approved for clinical use. Reformulation of drugs in liposomes has provided an opportunity to enhance the therapeutic indices of various agents mainly through alteration in their bio distribution. ¹.

The liposomes containing drugs can be administrated by many routes like intravenous, oral inhalation, local application, ocular and these can be used for the treatment of various diseases. A number of problems associated with drug molecule such as bioavailability, degradation, stability, site effect can be overcome by incorporating it into liposome. As a novel carrier system liposomes provide controlled and sustained release. There are a number of methods available by which liposomes can be manufactured separately depending on the property of molecule ².

The liposome drug delivery system shows advantages such as it helps to improve the bioavailability along with that it prolong residence contact time at the site of action, bioavailability of drug, it helps to provide sustained and controlled release of drug, reduces the dose frequency and improve the bioavailability. Liposome can prolong the drug action by slowly releasing the drug into the body³.

The current research topic undertaken to formulate and evaluate the liposomal based drug delivery system of Metformin HCl by using Thin film hydration technique, by using different Concentration of Phospholipids and Cholesterol to improve ultimately bioavailability of drug, improve Patient compliance and reduce the dose frequency.

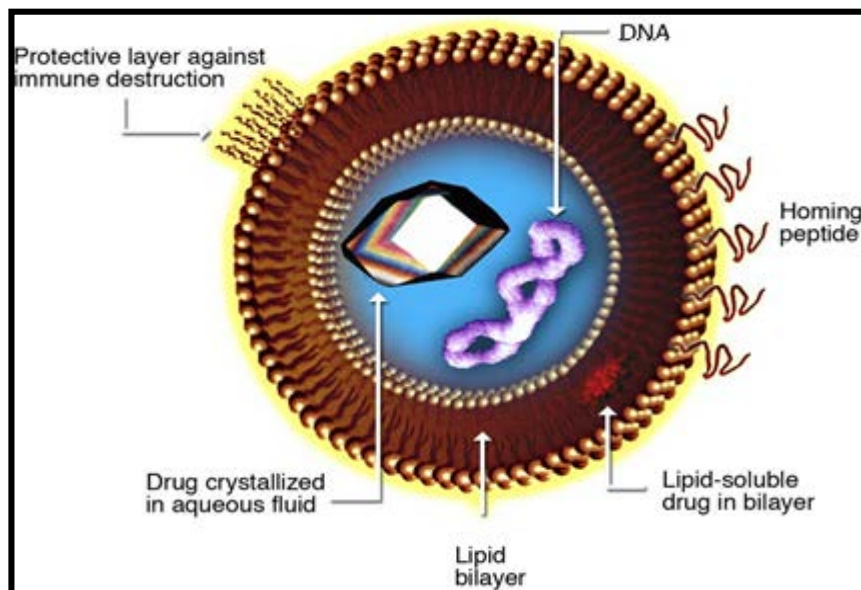


Figure 1:-Internal Structure of Liposome

MATERIALS AND METHODS

Metformin Hydrochloride was purchased from Yarrow Chem Products, Mumbai. Soya lecithin was purchased from Himedia laboratories Pvt.Ltd. Mumbai, Cholesterol was purchased from Loba. Chemie Pvt. Ltd. Mumbai. and other materials and solvents like Chloroform, Ethanol, Magnesium Stearate Microcrystalline Cellulose used were of analytical grades.

Rotary Evaporator instrument of Super fit Rotavap PBV-70, Centrifuge of REMI1MLH used for formulation and development of liposome. In vitro drug release study was performed using Nasal diffusion cell, UV Visible spectrophotometer shimadzu UV-1700, FTIR spectrophotometer Shimadzu 1700, Differential Scanning Calorimetry PerkinElemer 4000, Scanning Electron Microscopy (SEM) JSM-6360A.

Preparation of Metformin HCl liposomes by using thin film hydration technique

The liposomes were prepared by thin film hydration technique by using Rotary Evaporator instrument. The different formulation of Metformin HCl liposomes was prepared as shown in table no.1. Metformin HCl 500 mg, Soya lecithin (600mg, 700mg, 800 mg, 900 mg) and Cholesterol (400 mg, 300 mg, 200 mg, 100 mg) was dissolved in 8 ml of Chloroform and 2 ml of ethanol This solution was taken in a 250 ml round bottom flask. The flask was rotated in rotary flash evaporator at 80 rpm for 30 minutes in thermostatically controlled water bath at 40°C under vacuum 900 mm Hg. The organic solvent was slowly removed by this process such that a very thin film of dry lipids was formed on the inner surface of the flask. The dry lipid film was slowly hydrated with 10 ml of phosphate buffer pH 6.8 and rotated for 2 hr. swirling the contents to yield milky white suspension. The formulation is subjected to centrifugation at 3000 rpm for a period of 30 minutes. The liposome of Metformin HCl was obtained & further it has been considering for its characterization and stored in well closed container for further use.⁴



Figure 2: Rotary Evaporator Instrument used for Formulation and development of liposome

Table no 1: Formulation Batches of Metformin HCl Liposomes

Sr.no	Ingredients	Formulations			
		F1	F2	F3	F4
1.	Metformin HCl	500 mg	500 mg	500 mg	500 mg
2.	Soya lecithin	600 mg	700 mg	800mg	900mg
3.	Cholesterol	400mg	300 mg	200 mg	100 mg
4.	Phosphate buffer pH (6.8)	10 ml	10 ml	10 ml	10 ml
5.	Magnesium Stearate	5 mg	5 mg	5mg	5mg
6.	Microcrystalline Cellulose	2 mg	2mg	2mg	2mg
7.	Chloroform	8ml	8 ml	8 ml	8 ml
8.	Ethanol	2 ml	2ml	2 ml	2 ml

Evaluation of Prepared Liposomes:

Micrometrics Characteristics

The prepared formulation was evaluated for its Micromeritics Characteristics i.e. Angle of repose, Bulk density, Tapped density, Carr's index, and Hausner rati,result shown in table no 2^{5, 6}

Particle Size Determination:

Particle size of the liposomes was determined using optical microscopy technique. A standard stage micrometer was used to calibrate the eye-piece micrometer. Result shown in figure no 4^{7, 8}.

Determination of Entrapment Efficiency percentage

Drug entrapped within the liposomes was estimated after removing the untrapped drug, which was separated by collecting the supernatant after subjecting the dispersion to centrifugation in a cooling centrifuge at 3000 rpm at a temperature of 40°C for 30 min, where upon the pellets liposomes were washed again with buffer to remove any untrapped drug and the washing was combined with supernatant and was analyzed at 233nm. Result shown in figure 5^{9, 10}

$$\text{Entrapment Efficiency Percentage} = \frac{\text{Entrapped drug}}{\text{Total drug}} \times 100$$

Percentage yield

The prepared liposomes were collected and weighed. The measured weight was divided by the total amount of drug and ingredients which were used for the preparation of the liposomes. Result shown in figure 6^{11, 12}

$$\text{Percentage yield (\%)} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and excipient}} \times 100$$

Scanning Electron Microscopy (SEM)

The SEM studies for formulated liposomes were conducted by (JEOL and Tokyo, Japan JSM-6360), Pune university, Physics Department. The SEM are shown in figure 7

FTIR Spectroscopy

Fourier Transform Infra-Red Spectroscopy of formulations were recorded on Shimadzu Fourier Transform Infra-Red spectrophotometer using KBr powder. The instrument was operated under dry air purge and the scans were collected at scanning speed 2mm/sec with resolution of 4 cm^{-1} over region $4500\text{-}400\text{ cm}^{-1}$. The FT-IR spectra of polymer and complex formulation result Figure 9, 10, 11 and 12

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was performed for formulation on PerkinElmer 4000 instrument. Thermographs were obtained by heating 1 mg samples in aluminum pans at heating rate $100^\circ/\text{min}$, from 30°C to 350°C , in a nitrogen atmosphere (flow rate $20\text{ mL}/\text{min}$). Data was analyzed, using PYRIS Version-11.1.0.0488 software, for origin to obtain onset temperature (Tonset); the peak temperature (T peak) and the endset temperature (T endset) of endothermic peak. The DSC curve are shown in figure 13

In -Vitro Drug Release Studies

The small intestine of goat was collected from local slaughtering house. It was cleaned by normal saline water. Further it was used to tie at one end of donar chamber tube, for drug release study.

The in vitro drug release was carried out by using Nasal diffusion Cell through small intestine of goat. The release studies were carried out in 30 ml Phosphate buffer having pH 6.8 was placed in receiving chamber. The diffusion cell assembled on a magnetic stirrer at 30 rpm and the medium was equilibrated at $37\pm 50^\circ\text{C}$. Metformin hydrochloride liposome contain 500 mg of approximately dose was kept in donar chamber tube in diffusion cell and other end was closed. Then receiving chamber tube was inserted in phosphate buffer solution present in donar chamber. Aliquots were withdrawn (1 ml) at specific intervals, filtered and the apparatus was immediately replenished with same quantity of fresh buffer medium. The samples were filtered through Whatman filter paper No. 41. Samples were then analyzed at 233 nm using UV spectrophotometer (Model UV 1700, Schimadzu) with proper dilution. The % drug release was calculated. ¹³.



Figure 3 : Nasal diffusion cell assembly used in experimental work for drug release study

Release Kinetics and Mechanisms

The rate and mechanism of release of Metformin hydrochloride from the prepared Liposomes were analyzed by fitting the release data into zero order, first order, Higuchi and kros Meyer model. Result as represented in Figure14, 15,16 and 17 and Table no 3 ¹⁴.

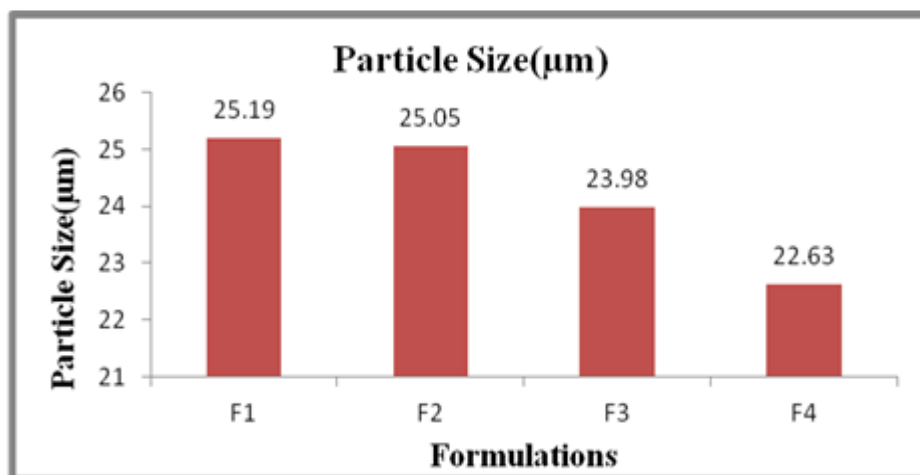
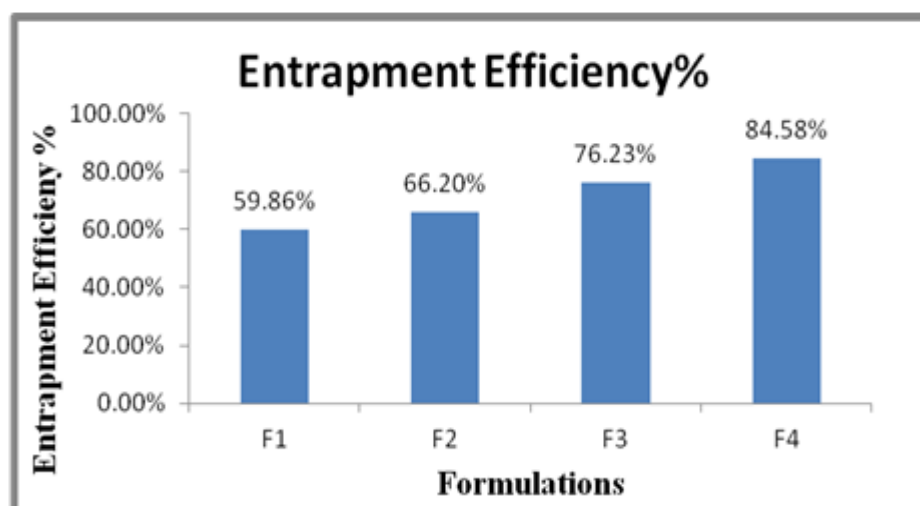
Accelearted Stability Studies:

The Metformin HCL liposomes were subjected to Accelerated stability studies at elevated temperature 40°C , Room temperature and 75% Relative Humidity for days on the all formulations and result as represented in Figure no 18 ¹⁵.

Observation And Evalution Of Liposomes:

Table no. 2 :- Micrometrics characteristics of liposomes

Sr. no.	Batch	Mean Bulk Density (gm/ml) \pm SD	Mean Tap Density (gm/ml) \pm SD	Mean Hausner Ratio \pm SD	Mean% Compressibility Index \pm SD	MeanAngle of repose ($^{\circ}$) \pm SD
1	F1	0.35 \pm 0.023	0.40 \pm .005	1.14 \pm 0.005	12.5 \pm 0.225	27.11 \pm 0.005
2	F2	0.47 \pm 0.005	0.53 \pm 0.01	1.11 \pm 0.017	11.32 \pm 0.121	30.11 \pm 0.01
3	F3	0.45 \pm 0.01	0.50 \pm 0.005	1.12 \pm 0.015	10.0 \pm 0.1154	28.81 \pm 0.005
4	F4	0.35 \pm 0.005	0.43 \pm 0.017	1.22 \pm 0.020	18.6 \pm 0.346	25.96 \pm 0.005

**Figure 4:- Graph of Particle Size (μm)****Figure 5:- Graph of Entrapment Efficiency %**

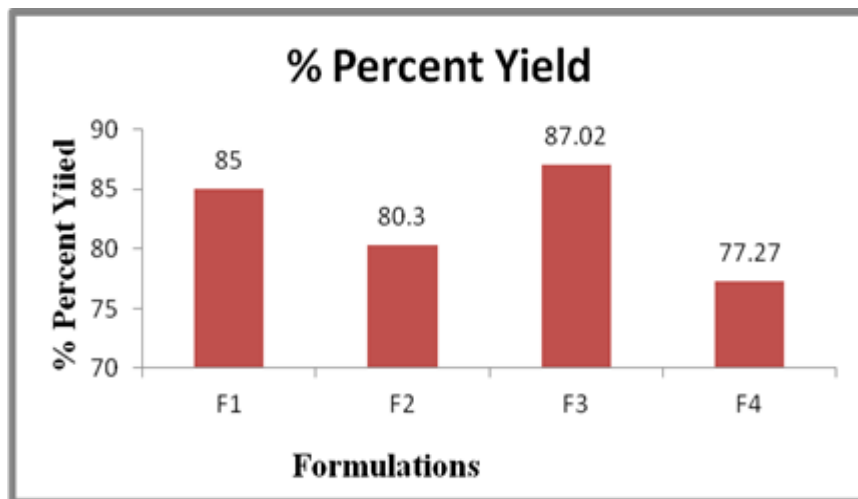


Figure 6:-Graph of % Percentage Yield

Scanning Electron Microscopy:

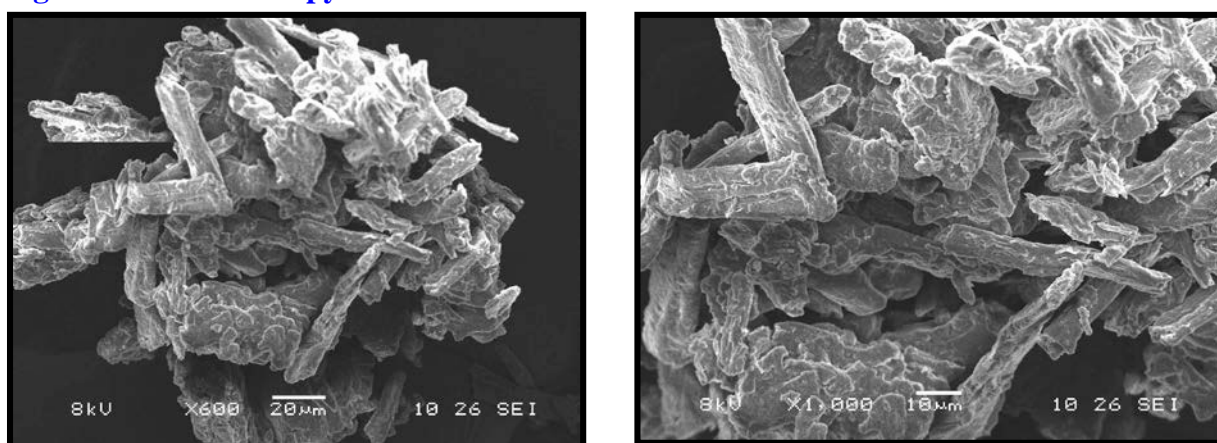


Figure 7:- Structure of Liposomes

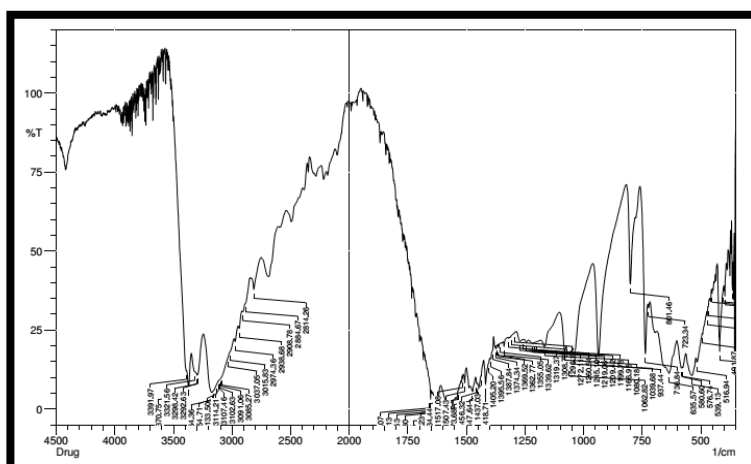


Figure 8:- FTIR spectra of Metformin HCl

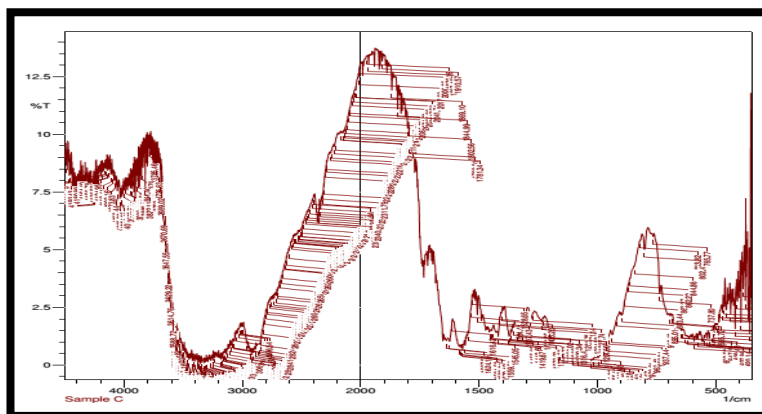


Figure 9 :- FTIR spectra of F1 Formulation

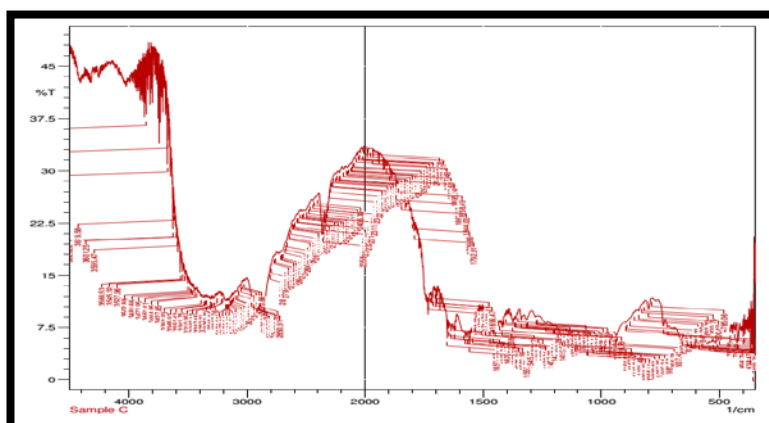


Figure 10:- FTIR spectra of F2 Formulation

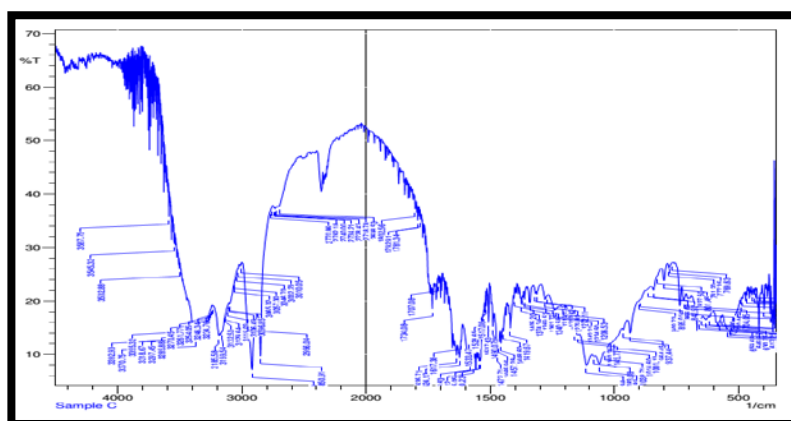


Figure 11:- FTIR spectra of F3 Formulation

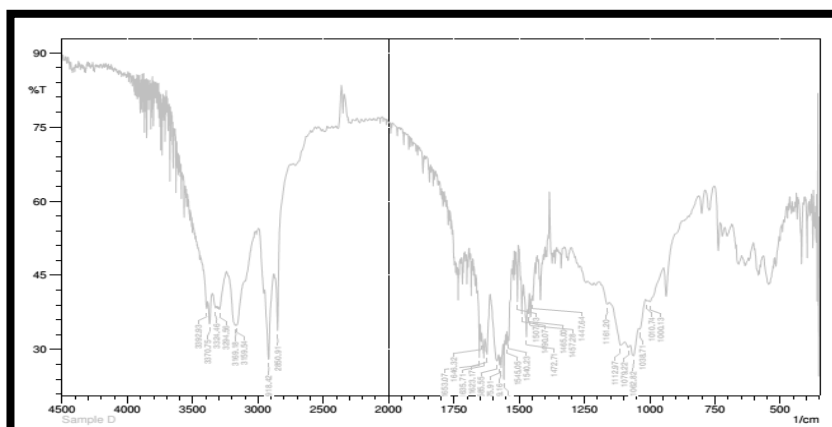


Figure 12:- FTIR spectra of F4 Formulation

Differential Scanning Calorimetry:

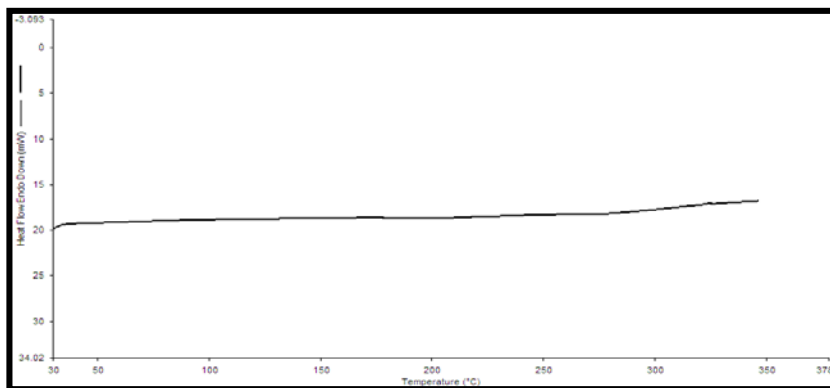


Figure 13: -DSC thermogram of formulation F1

In Vitro Drug Release Study:

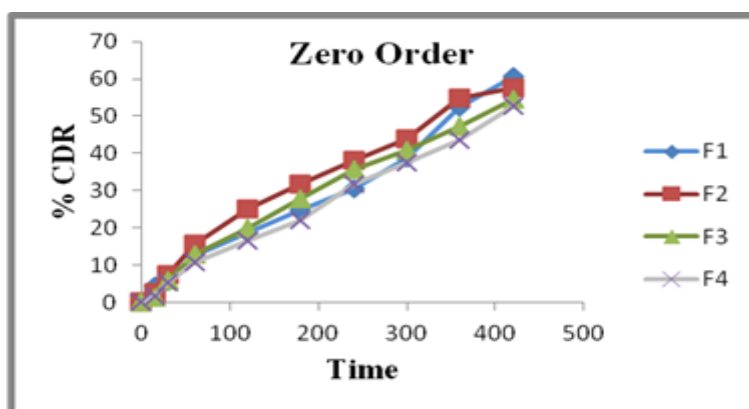


Figure 14:- Graph of cumulative percent drug release vs Time for batch F1-F4

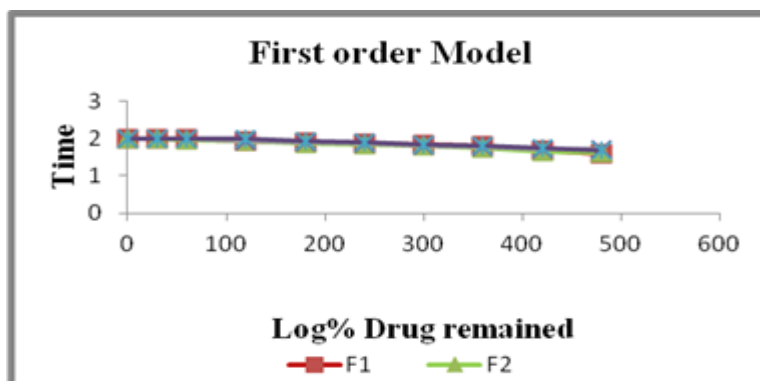


Figure 15:- Graph of Log % drug remained vs. Time for batch F1-F4

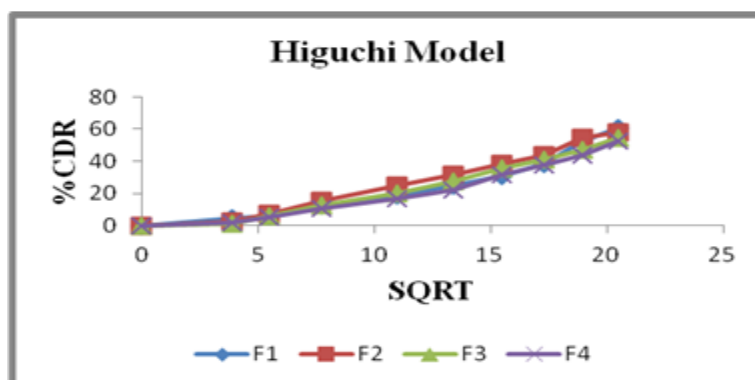


Figure 16:- Graph of cumulative percent drug release vs. Square root of time for batch F1-F4

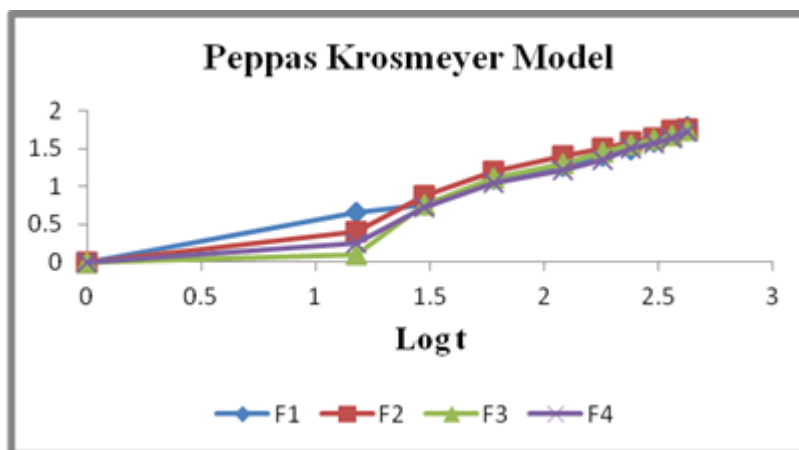


Figure 17:- Graph of Log percent drug release vs. Log of time for batch F1-F4

Drug Release Mechanism:

Table no. 3:- Kinetic assessment of drug release from liposomes formulation

Sr.No	Batch	Zero order	First Order	Higuchi	Peppas Krosmeier	
		R ²	R ²	R ²	R ²	N
1.	F1	0.987	0.9567	0.921	0.98	0.679
2.	F2	0.977	0.9914	0.976	0.899	0.082
3.	F3	0.987	0.9946	0.969	0.894	0.087
4.	F4	0.995	0.981	0.947	0.935	0.709

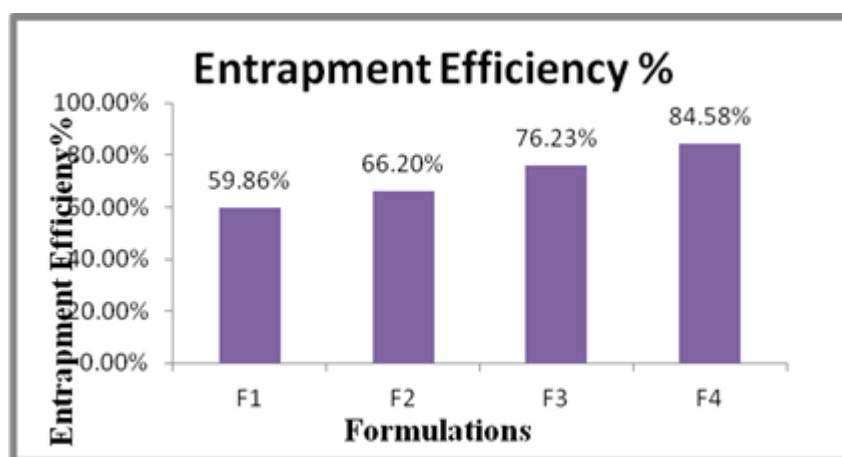


Figure 18:- Graph of Entrapment Efficiency % of formulation after 30 days

RESULT

Evaluation of Liposomes:

The Metformin HCL liposomes were successfully formulated by thin film hydration technique. A total of four batches were formulated by taking different concentration Cholesterol and Phospholipid. These liposomes were characterized for its Micrometrics characteristics, Particle Size, Entrapment Efficiency %, Percentage yield, SEM, FTIR study, DSC, In vitro drug release studies and Accelerated Stability Studies.

Micrometrics characteristics of liposomes:

The values of bulk density, tapped density, Carr's index, Hausner ratio and angle of repose were calculated as represented in table no.2

Bulk Density and Tapped Density

The bulk density and tap density of formulations was found to be 0.35g/ml to 0.47g/ml and 0.43g/ml to 0.50g/ml respectively.

Hausner Ratio

The Hausner ratio of all formulations <1.25 indicate good flow ability.

Compressibility Index

The compressibility index of formulations is in range of 10.00%-18.6% indicating the good flow properties.

Angle of Repose

The angle of repose of formulations ranges from 25.96° to 30.11° is indicating acceptable flow properties.

Particle Size Determination

Particle size of the Metformin HCl liposomes were evaluated by the optical microscopy and average particle size of all four formulation was calculated, mean particle size range for formulation was found to be 25.19 µm, 25.05 µm, 23.98 µm, and 22.63 µm respectively for F1, F2, F3 and F4 formulation respectively as represented in figure 4

It was noticed that the mean particle size increase with increases in cholesterol concentration might have occurred due to the fact that as cholesterol concentration increases, it produces a significant increase in the rigidity in a liposome, thus leading to an increase the size and finally a higher liposomes size.

Entrapment Efficiency %:

The entrapment efficiency % of the liposomes was found to be in the range between 61.43% to 88.24 % as as represented in figure 5 The F4 formulation showed the highest entrapment efficiency % of 88.24% and lowest entrapment efficacy of 61.43% was found in F1 formulation, while all other formulation showed entrapment between these formulations.

From this it is concluded that drug entrapment efficiency % increases with increase in the concentration of Soya lecithin.

Percentage Yield:

The percentage yield of different formulations (F1, F2, F3 and F4) were calculated and found to be 85%, 80.30%, 87.02%, and 77.27% respectively. The result of all four formulations of Metformin HCl liposomes as represented in figure 6

Scanning Electron Microscopy (SEM):

The microscopic properties like Structure of liposomes, Topography and magnification was evaluated by using scanning electron microscopy. Scanning electron photomicrographs of formulation as represented in figure 7

The liposomes were somewhat in a spherical structure with rough surface morphology and exhibited a range of sizes within each batch. Very less particulate matter of drug were seen on the surface of the liposomes that indicating uniform distribution of drug in polymer network.

Fourier Transform Infra-Red Spectroscopy (FTIR):

FTIR spectra of formulations showed the characteristics peak of Soya lecithin and Cholesterol. The observed spectrum represent drug to phospholipid super imposed pattern with their significant functional group at specific wave length indicated in its pure form. The spectra represents significant Functional groups C-N stretching H bond, CH₃ Stretching, C-C Stretching (in ring), C=C Stretching (in ring), O-H Stretching H bonded, N-H primary Stretching, N-H secondary Stretching. From FTIR Study no addition consequent peak is observed indicated that there is only physical interaction between drug to Phospholipid and there is absence of chemical interaction between drug to Phospholipid as represented in figure 9,10,11 and 12

Differential Scanning Calorimetry (DSC):

The DSC study of optimized formulation was done. There is no formation of endotherm as well as exotherm in this study. This may be happen due to degradation of drug sample. Though it is amorphous in nature as represented in Figure 13

In Vitro- Drug Release Study:

Diffusion studies on all the four formulation of Metformin HCL liposomes were carried out by using Nasal diffusion cell in 6.8 pH phosphate buffer as a medium and result revealed that drug release was followed a controlled manner for 7 hours.

The in-vitro release data obtained for formulation F1, F2, F3 and F4 as represented in Figure no.14,15,16 and 17 respectively. The cumulative percent drug release after 7 hrs was found 60.57 %, 57.65 %, 54.51 % and 52.7256 % up to 7 hr for formulation F1, F2, F3 and F4 respectively. The results obtained shows that the drug release was lowest 52.72% for F4 formulation while highest drug release 60.57 % for F1 formulation.

Kinetic model for drug release:

The release kinetics of formulation was determined by various models like Zero-order model, First order model, Higuchi's (Matrix) model, Peppas Korsmeyer model. The best fitting model was determined from the

regression coefficient (R^2) and release exponent (n).The formulation F1 and F4 followed Zero order release model and F2 and F3 followed First order release model.

The regression coefficient (R^2) and release exponent (n) of all formulation were reported in the table no.3 The values n were greater than 0.5 and less than 1 hence the formulation follows the Non-Fickian diffusion release.

Accelerated Stability Studies:

The Metformin HCL liposomes were subjected to Accelerated stability studies at elevated temperature 40°C, Room temperature and 75% Relative Humidity for days on the all formulations and result as represented in figure 18. Liposomes were observed for any change in particle size and entrapment efficiency %. The liposomes did not show any significant change in colour and entrapment efficiency % at the end of 30 days. The stability result was indicated that all the formulations was stable for 30 days.

CONCLUSION

The prepared liposomal formulations using different lipid cholesterol molar ratios were developed and evaluated. The evaluation studies concluded that higher the concentration of cholesterol (phospholipid: cholesterol = 6: 4) rigidity of the bilayer will be more and showed better controlled release of Metformin hydrochloride. Hence the combination (6:4) used in F1 is the optimum for achieving better controlled release and liposomal formulation of Metformin hydrochloride would be a suitable means to produce more uniform release profile.

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