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FORMULATION & EVALUATION OF PROLIPOSOME OF MORIN BY USING SOLVENT EVAPORATION TECHNIQUE

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ABSTRACT

The purpose of this study to develop and evaluate Morin pro- liposomes powder oral drug delivery system. Pro-liposomes are phospholipid-based drug delivery system. Proliposomes prepared using solvent evaporation technique by varying lipid phase (phosphatidylcholine/ cholesterol) and Mannitol composition. It promotes drug absorption, increases the efficacy and therapeutic index of drug and also increases stability via encapsulation, biocompatible, biodegradable and non-toxic. Proliposomes formulation was characterized for entrapment efficiency, drug content, surface morphology, number of vesicles. Followed by In-vitro release studies. Oral pro-liposomes powder showed good properties with angle of repose was less than 30, Car's index and Hausner's ratio were also less than 21 and 1.25, respectively. The mean size of the vesicle was in the range 200-300 nm. The assay and entrapment efficiency of pro-liposomes formulation were 57.66±0.1 to 96.79±0.4%, respectively. In vitro release of Morin proliposome powder formulation was 96.15 after 24 hrs. which shows good release from the vesicle of proliposome. In vitro studies carried out provides an insight on the stability and enhances dissolution of from proliposome formulation. The solid state characterization like SEM, FT-IR, DSC studies of Morin Proliposome were carried out.

KEYWORDS: Morin, phosphatidylcholine**,** Box-Behnken design.

INTRODUCTION

Morin (3, 5, 7, 2′, 4′-pentahydroxyflavone) is one of the flavonols that has been identified in fruits, vegetables, tea, and many medicinal herbs from Asia. It has been reported to possess anti-inflammatory, anticancer, antioxidant, antihypertensive, and anticlastogenic activities.[1–2] It has also been found to have an inhibitory effect on xanthine oxidase. The available pharmacokinetic data in both humans and rats are scarce at present.[3–5] A previous study compared the pharmacokinetics of morin after oral administration but failed to consider the absolute bioavailability of morin.[6-8] As a poorly water-soluble drug, the absolute bioavailability of morin after a single oral dose is very low. Low oral bioavailability may result in decreased efficacy when therapeutic plasma levels are not achieved, or may result in unanticipated toxicity at a high dose of morin.[2–6] However, to date, few studies have focused on the applicable formulation of morin through oral administration. Most studies have used a simple morin aqueous solution via parenteral route to obtain the targeted pharmacological effect. Parenteral administration offers the best absorption but has obvious disadvantages, such as low patient compliance, safety considerations, and high medication costs. Thus, there is a pressing need to develop a new oral dosage form of morin with improved bioavailability and furthermore to obtain successful therapeutic effects at a decreased oral dose.[8-10]

Oral delivery is the most convenient route of delivery due to its ease of administration, high patient's compliance, cost effectiveness, less invasive and flexibility in the design of dosage form. The potential approach was developed to address their major issue related to the oral delivery system. The oral proliposome formulation of a drug depends on several factors such as solubility in water, dissolution rate, and permeability across gastro-intestinal (GI) membrane, first-pass metabolism, GI stability, and vulnarability to efflux mechanisms. The common causes for low bioavailability following oral administration are attributed to the drug's poor solubility and high first pass metabolism. Proliposome can be defined as dry, free flowing powder with mannitol surface as a base to adsorb the liposome suspension.[11-15] Liposome are the lipid carrier containing phospholipid bilayers with cholesterol as a membrane stabilizer. The major advantage of the oral proliposome powder is avoid fusion, hydrolysis, and aggregation Oral proliposome powder is a lipid-based system that has property to improve the oral absorption of the drug which can be valuable for drug having

narrow therapeutic window[16-18]. These oral proliposome powder is the suitable oral drug delivery system to improve the physicochemical as well as pharmacokinetics performance of the drugs.[19-20]

The aim of the relevant research work was to develop proliposomes formulation for the enhancement in the efficient and effective delivery of morin as active moiety. This study is potential approach to improvise the poor solubility, low permeability due to the P-gp Efflux and the low oral absorption of the morin. The proliposomes formulation was to develop by encapsulating the morin inside the nanocarrier which has lipid bilayer and may cross the lipid membrane in the body and releases the morin at the site of action.

MATERIAL AND METHODS

Material

Morin was purchased from TCI chemical (India) Pvt .Ltd., Methanol and Chloroform were purchased from Fisher scientific, Mumbai, India. Phosphatidyl choline (Soya lecithin) was purchased from HIMEDIA Laboratories, Mumbai, India, cholesterol was purchased from Finar Reagents, Mumbai, India, mannitol were purchased from Sd Fine-Chem Ltd, Mumbai, India, acetonitrile was purchased from TCI Chemicals Pvt. Ltd, India. Rotary vacuum evaporator was from Heidolph instruments. All chemicals used were of analytical grade, and solvents were of HPLC grade. Freshly collected double-distilled water was used throughout the experiments.

Methods

Preparation of proliposome

Proliposome of Morin was formulated using solvent evaporation technique. Accurately weighed amounts of lipid mixture comprising of phosphatidyl choline (soya lecithin) and cholesterol at various molar ratio as shown in Table 1 and were dissolved in 20 ml of solvents mixture containing chloroform and methanol in the ratio 1:1. The suspension containing phosphatidyl choline, Morin, cholesterol, and mannitol was transferred in to round bottom flask. All material got dissolved in solvent mixture, except carrier material; hence resultant suspension was obtained like slurry due to the addition of mannitol act as base carrier. The organic solvent mixture was evaporated with the help of rotary vacuum evaporator under the reduce pressure 50mbar at the temperature of 40 ± 2 °C to 55 ± 2 °C. After ensuring the removal of solvent, the resultant powder was further dried in a vacuum desiccator at room temperature so as to obtain dry, free flowing powder. This powder was stored in the tightly closed container.

Experimental design

Experimental statistical design which includes the surface response methodology was used for optimizing the morin loaded oral proliposome powder formulation and evaluating the different effects including main effects, interaction effects, and quadratic effects of the formulation components on the entrapment efficiency of morin loaded oral proliposome powder. The three-factor, three-level designs were generated using Design Expert (Version 8.7.0.1 Stat-Ease, Minneapolis, MN). For this study, the Box–Behnken design was chosen because it requires fewer runs than other design in case of three or four variables. This design of the morin loaded oral proliposome powder formulation comprising 15 runs was developed. The independent variables include the ratios of lipid, drug, and mannitol are presented in Table 1 along with their low, medium, and high levels, which were selected based on the results from preliminary batches of morin loaded oral proliposome powder formulation whereas, concentration range of Lipid: Lipid ratio (A), Lipid: Drug ratio (B), and Lipid: Mannitol ratio (C) used to prepare the 15 formulations design and the respective observed responses are given in Table 1&2.

Table 1: Variables and Experimental Design levels for Morin Proliposome.

Independent variables	Coded symbols	Levels		
		-1	0	+1
Lipid: lipid ratio (w/w)	A	0.5:1	1:1	1:0.5
Lipid: drug ratio (w/w)	B	1:1	1:2	1:3
Lipid: mannitol ratio (w/w)	C	1:10	1:50	1:90

Table 2: Experimental Design of batch 1-15.

Batch Code	A	B	C
B1	+1	-1	0
B2	-1	-1	0
B3	+1	+1	0
B4	-1	+1	0
B5	-1	0	-1
B6	-1	0	+1
B7	+1	0	+1
B8	+1	0	-1
B9	0	-1	-1
B10	0	+1	-1
B11	0	-1	+1
B12	0	+1	+1
B13	0	0	0
B14	0	0	0
B15	0	0	0

EVALUATION OF PROLIPOSOME

Flow properties of proliposome

Flow properties or rheological studies of the were characterized by measuring the angle of repose, Car's index, and Hausner's ratio. The angle of repose was by the fixed funnel method whereas Car's index and Hausner's ratio were calculated from the bulk and tapped density of proliposome powder.

Determination of vesicle size/particle and number of vesicles per mg proliposome

1mg of proliposome powder was accurately weighed. A drop of distilled water was added to this powder on glass slide without cover slip. It was observed for formation of liposome from proliposome formulation. Vesicle size and count was recorded under Digital optical microscope [DMWBI-223ASC, motic] with magnification 10x.

Drug content

The morin content was determined by dissolving the proliposome powder equivalent to the 1000 µg of drug in 1ml of Methanol. The aliquot sample was taken in to microcentrifuge tube and vortexed for 30 sec. The microcentrifuge tube was sonicated for 2 min. ensuring that all lumps are broken. It was centrifuged at 10,000 rpm for 15 min at RT. The 500µl of supernatant liquid was collected and transferred in to labeled 5ml volumetric flask. The volume was made up to 5ml using Methanol and analyzed with UV spectrophotometer at wavelength of 254 nm.

Entrapment efficiency

The entrapment efficiency of proliposome formulation was determined by dissolving morin proliposome powder equivalent to 1000 µg of drug in 1 ml of methanol. It was vortexed for 30 sec. and then centrifuge at 10,000 rpm for 15 min at RT. The 500µl supernatant liquid was collected and transferred into labeled 5ml volumetric flask. The volume was made up to 5ml using methanol and analyzed with UV spectrophotometer at 220 nm.

In vitro dissolution study

In vitro dissolution study of Morin Proliposome was performed using USP type I Dissolution testing apparatus fitted with paddles. The speed of rotation of paddle was set at 50 rpm. Dissolution study was carried out using 500ml of phosphate buffer (pH 6.8) Maintained at a temp of 37°± 5°C under sink condition. Proliposome powder equivalent to 50 mg of drug was added to the dissolution medium. At a predetermined time intervals of 5, 10,15,30,45 min. for first hours and for next 24 hours samples were collected. 5 ml samples were withdrawn, filtered through Whatman filter paper, and analyzed by UV spectrophotometer at 220 nm. Drug content was determined by placing absorbance in standard curve.

Scanning electron microscopy (SEM)

The surface morphology of the proliposome powder was investigated by scanning electron microscope (SEM) (JSM-6510, JEOL). Sufficient amount of formulation was weighed and mounted on the stub. This specimen was then coated with platinum particles and observed with scanning electron microscope. Images were recorded using the back scattered electron (BSE) compositional signal with an accelerating voltage of 5KV.

Fourier transform infrared spectroscopy (FT-IR)

After starting the instrument, Background Measurement was performed without placing the drug on panel. For Sample Measurement', pure Morin was placed on cleaned panel of FT-IR Spectrometer (Bruker). The placed Morin was sandwiched between panel and upper arm. This sample was scanned over a wave number range of 4000 to 500 cm-1. Similar procedure was followed for Phosphatidyl choline, Cholesterol, Mannitol, blank proliposomes, batch B1 and optimized batch.

Stability studies

Proliposome stability study was performed as per the method reported previously The Proliposome formulation stored in glass vials were covered with aluminum foil and kept at room temperature and refrigerator at 4±0.2°C for a period of 1 month. After 1-month samples were withdrawn and hydrated with distilled water and observed for any sign of drug crystallization under optical microscope and evaluated for colour & Entrapment.

RESULT AND DISCUSSION

Flow properties

Flow properties is an very important evaluation parameter for proliposome powder. There are three flow properties can be used to evaluate the powder formulation mainly three acceptable range for angle of repose is (<30)°, Hauser's ratio is (<1.25), Car's index is (<21). The optimizes batch A showed all flow properties value like angle of repose (29±0.5), Hauser's (1.11±0.5)ratio, Car's index (12.23±0.8) was acceptable range. Flow properties of all batches as well as and optimized batch are indicated in Table 3.

Table 3: Flow Properties of Proliposome Formulation.

Batch code	Angle of repose (0)	Car's Index	Hausner's ratio	Vesicle size/particle size	%Drug Content	%Entrapment efficiency
B1	25±0.4	20.04±0.5	1.13 ±0.4	125.1±0.2	65.15±0.1	68.38±0.1
B2	28±0.7	18.94±0.6	1.28±0.6	137±0.5	79.15±o.3	79.33±0.8
B3	35 ±0.2	19.04±0.5	1.13 ±0.7	125±0.3	89.66±0.2	88.15±0.3
B4	34 ±0.3	16.23 ±0.5	1.25±0.1	101.6±0.2	56.22±0.1	65.85±0.6
B5	31 ±0.5	22.05±0.5	1.18 ±0.3	147.2±0.6	57.66±0.6	63.15±0.5
B6	30±0.7	20.73 ±0.4	1.17 ±0.5	125.1±0.2	73.75±0.3	57.66±0.1
B7	25±o.6	19.38±0.2	1.24 ±0.6	116.5 ±0.4	75.65±0.3	72.75±0.1
B8	28 ±0.3	20.58±0.6	1.19 ±0.8	118.6±0.4	78.33±0.6	75.65±0.5
B9	31±o.5	12.94±0.6	1.15 ±0.9	128.2±0.5	89.33±0.6	81.25±0.2

B10	2330.3	13.23±0.3	1.14 ±0.9	149.5±0.2	61.58±0.1	87.15±0.3
B11	33±0.8	12.50±0.7	1.18 ±0.8	138.5±0.1	94.25±0.1	71.58±0.2
B12	29±0.5	12.23±0.8	1.11±0.5	148.4±0.8	72.25±0.5	96.79±0.4
B13	28±0.8	13.23±0.7	1.15 ±0.4	107.6±0.3	74.25±0.3	95.89±0.7
B14	27±0.4	17.74±0.4	1.22 ±0.2	126.5±0.4	85.25±0.2	75.25±0.1
B15	23 ±0.6	17.43±0.6	1.31±0.6	112.5±0.1	74.25±0.3	84.25±0.4
MB-A	29±0.5	12.23±0.8	1.11±0.5	148.4±0.8	78.25±0.5	96.79±0.4

Vesicle size/particle size number of vesicle

Vesicle size or particle play a significant role in the vesicular system. The main size of vesicles was in the range of 200-300nm. The proliposome formulation vesicle images figure1. After hydration of proliposome powder captured by the optical microscopy software. The mean average particle size of vesicle of batch B-1 to B-15 was found to be in the range of 200-300nm. The optimized batch A showed the optimum particle were distributed in the range of 161±0.5nm (Figure.1) .the vesicle size of formed liposome is depending on the concentration of the different parameter. the concentration of cholesterol increased, the size of the vesicle increased. Increased concentration of the lipid phosphatidylcholine play important role on forming the maximum bilayer formulation that finally increase the number of liposome vesicles.

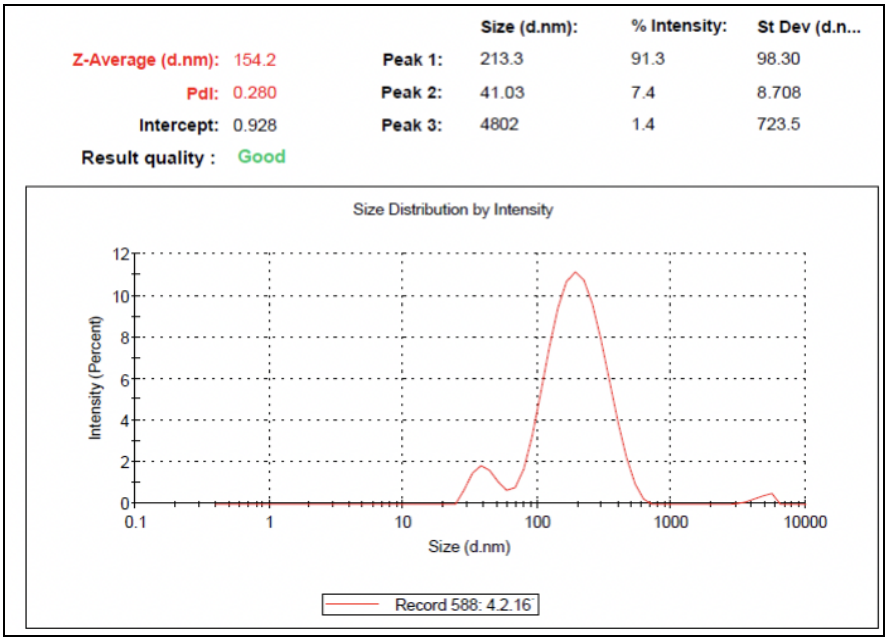

Figure 1: Particle size optimized Morin Proliposome.

Drug content and Entrapment efficiency

Proliposome formulation was evaluated for its drug contents analysis. From analysis study, it was observed that total drug content in the formulation of B-01 to B-15 was obtained in the range of 65-89%. The total drug content of optimized batch A was obtained 78.25%and also entrapment efficiency was found to be 96.79% which is shown in Table 3.The increase or decrease in the entrapment efficiency of formulation is totally dependent on the composition of the proliposome powder formulation .The concentration of the cholesterol and phosphatidylcholine plays the key role in the entrapment of the drug like morin in the liposome vesicle. Due to the hydrophobic nature of Morin, it will get more entrapped in the hydrophobic moiety of liposome. Increase in the concentration of cholesterol also results in the entrapment of Morin in the liposome vesicle.

In vitro dissolution study

In vitro drug release study proliposome powder was carried for 24 hrs. Initially in the release study, the mannitol was solubilize in the dissolution media. The in vitro drug release study for morin was carried out at pH 6.7 due to Morin at this pH. Also it is to be important that, the proliposome powder should be freely dispersed in the media to from liposome suspension. So, by adding the buffer and maintain it at pH 6.7 will increase the solubility directly increased. The proliposome formulation of batch B-1 to B-15 was ranging of 72% to 96%v showed in figure 2-4. The total percent release of optimized A and dissolution profile of optimized batch A was found to be 96.15% showed in figure 5. To ensure the effect of the different composition vesicle was studied. Initially, the rapid release of the Morin was observed. This may be due to the unentrapped drug which may be present in the hydrophobic region of the

liposome. The unentrapped Morin will be released in the dissolution media rapidly.

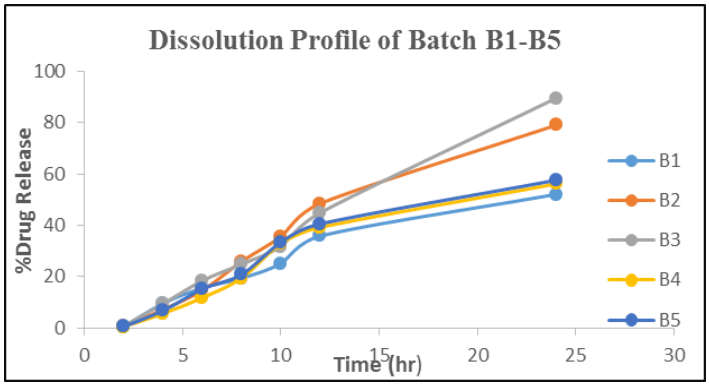

Figure 2: Dissolution Profile of Batch B1-B5.

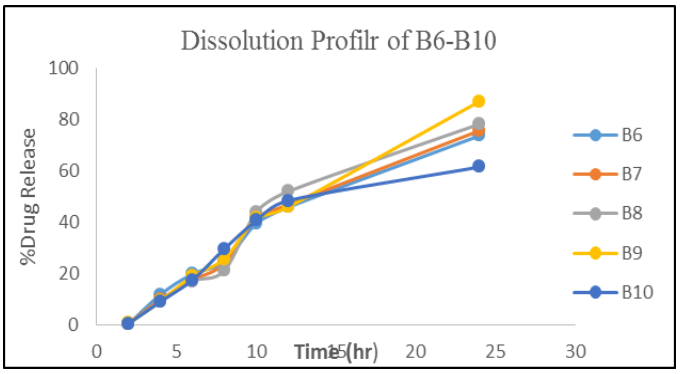

Figure 3: Dissolution Profile of Batch B6-B10.

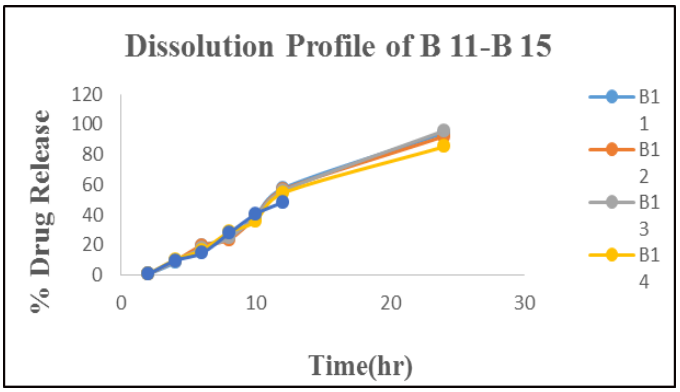

Figure 4: Dissolution Profile of Batch B11-B15.

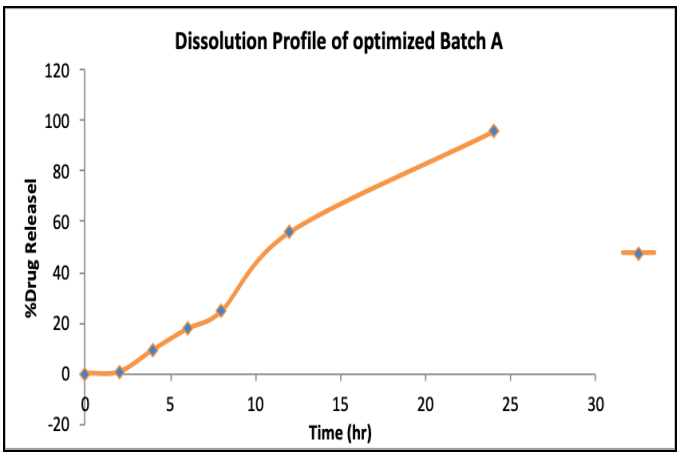

Figure 5: Dissolution optimized batch A.

Scanning electron microscopy (SEM)

The surface morphology of the carrier material like mannitol Figure.6 (A) and optimized batch A Figure (6 B) (MB-A) were evaluated by SEM, shows the absence of the crystals of the drug material on the surface of the proliposome formulation. The absence of the morin crystal shows the drug was not leached out from the vesicle of the liposome. It is totally entrapped in the bilayer structure of the liposome or lipophilic region of the liposome. It also indicates the uniform distribution of the drug-like morin within the core of proliposome. Deposition of the phospholipid on carrier material indicates the formation pf proliposome.

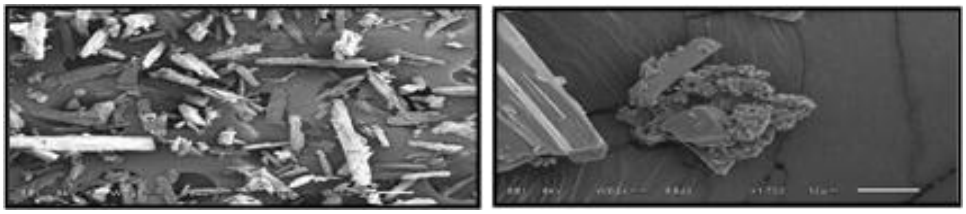

Figure 6: SEM Images: (A) Mannitol (B) Proliposomes Formulation.

Fourier transform infrared spectroscopy (FI-IR)

From the FT-IR spectra of the morin Figure 7, phosphatidylcholine, cholesterol, physical mixture, and optimized batch A Figure 8 It was obsered that, there was no interaction the entrapped drug and other ingredients.

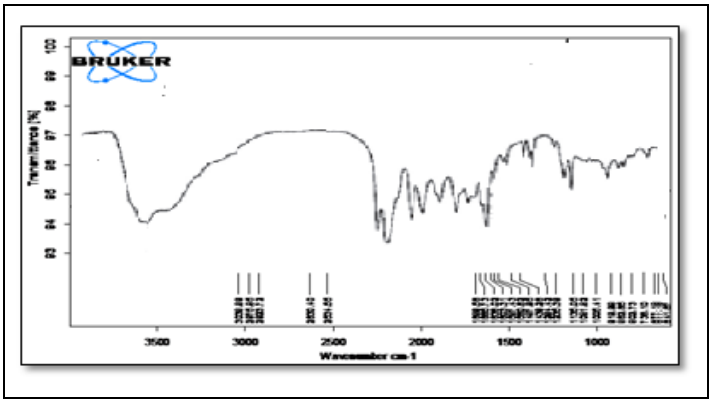

Figure 7: FTIR Spectra of Morin.

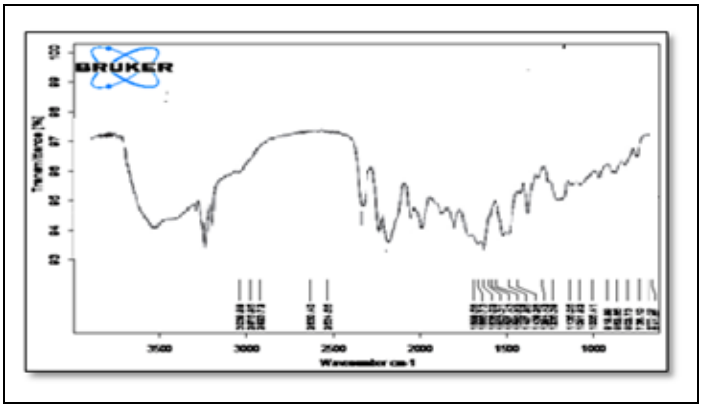

Figure 8: FTIR Spectra of Proliposome Optimize Batch.

Differential scanning colorimetry

In the DSC plot of morin the sharp curve was observed at 287.86 ˚C showed the melting of the morinn which is match with the melting point 287 ˚C given the previous reports. In this shown Figure 9.

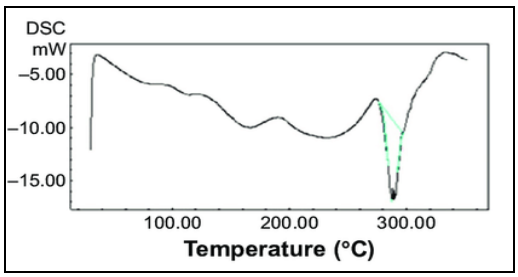

Figure 9: DSC of Morin.

Stability study

Proliposome optimized batch A was kept for stability at (4±2°C and room temperature for 1 month observed for any drug crystallization under microscope and evaluated for color, and entrapment efficiency. Stability parameter of proliposome formulation was given in Table 4.

Table 4 Stability parameter of proliposome optimized batch A

Sr No.	Temperature	Particle size (nm)	Entrapment
1	4±	137±5	96.89±0.2
2	RT	140±2	92.05±0.5

Analysis of data by design expert software

Design expert was selected and the 3 factors were evaluated at 3 levels, respectively. The Lipid: Lipid ratio (A), Lipid: Drug ratio (B) and Lipid: Mannitol ratio (C) was selected as independent variables and the dependent variable was Entrapment efficiency. The data obtained were treated using Stat Ease Design Expert 8.0.7.1 software and analyzed statistically using ANOVA. The data were also subjected to 3D response surface methodology to study the interaction of as Lipid: Lipid ratio (A), Lipid: Drug ratio (B) and Lipid: Mannitol ratio (C) as dependent variables shown in figure 10. On the basis of Entrapment efficiency of the batches prepared as per experimental design, we optimized the experimental design levels for variables.

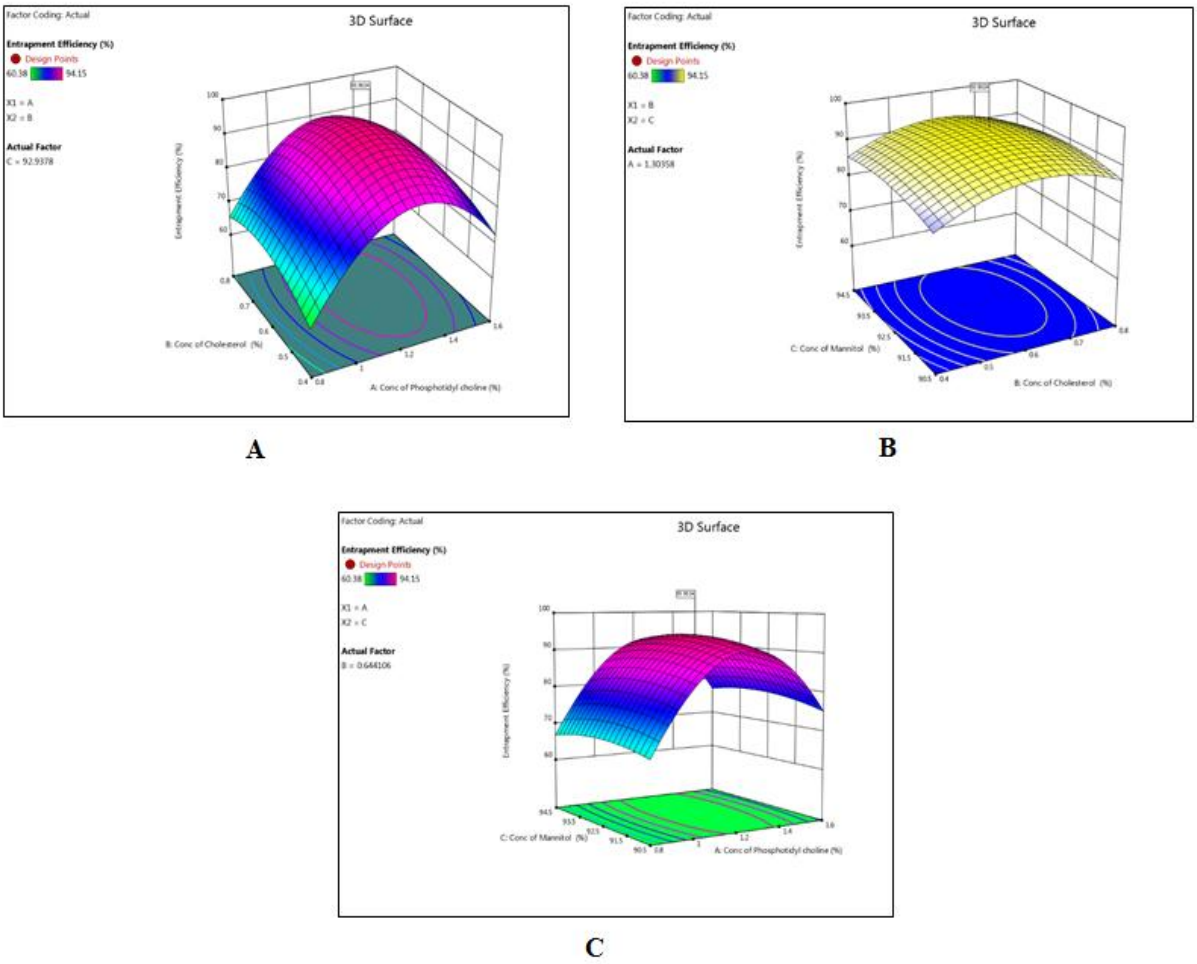

Figure 10: Response surface curve (A) lipid: drug ratio, (B) lipid: mannitol ratio & (C) lipid: lipid ratio.

Result response Entrapment efficiency was 96.79% showed by design expert software which was close to the percent entrapment of theoretical batch 95.18±0.5, i.e. optimized batch. In that showed that, a very good fit to the mathematical model. When the different factor and an interaction between variable have p-value less than 0.05, it states about the process way in a significant way. The analysis of variance showed that this regression model was highly significant with p-value less than 0.05 Quadratic model was suggested by the software. The adjusted R2 value of model was found to be 0.9576 which states that 2.47% of the variation in the results were not explained by the given model. It was observed that, concentration of cholesterol: mannitol (B) variable

has the largest effect on the entrapment efficiency as it states significant <0.0001 p-value whereas, Cholesterol and Phosphatidyl choline (A) and Mannitol and Phosphatidyl choline (C) variable interaction was significant with at <0.01 p value. Also, BC variable interaction was significant with small p-value. Morin proliposome formulation is influenced by the increasing the solubility of components. But it is depend upon the amount or concentration of each component in the formulation.

CONCLUSION

The present research study report was formulation development and evaluation of Proliposome containing Morin as a model. The proliposome was successfully prepared by using solvent evaporation technique. Phosphatidylcholine and cholesterol mannitol is using a carrier for proliposome formulation it possesses high porosity, surface area. The Morin could be loaded into proliposome with increase the drug content, entrapment efficiency and drug stability issues. In vitro release study showed significant increase in the drug profile of optimized batch (MB-A) containing Morin.

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