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Formulation and Evaluation of Metoprolol Tartrate Loaded Niosomes Using 2^3 Factorial Design

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Authors' contributions

This work was carried out in collaboration between all authors. Author SN designed the study. Authors SIK and WN managed the analyses of the study. Author OH managed the literature researches. Author ID wrote the first draft of the manuscript and wrote the protocol. Author SMAI performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of this study was to formulate and evaluate metoprolol tartrate (MT) loaded liposomes using factorial design. Preparation of niosomal drug delivery of MT increased its bioavailability which led to being better therapeutic effects, reduced the frequency of dosing and decreased side effects of hypertensive patients. Ether injection method (EIM) and thin film hydration method (TFHM) were used for the preparation of all formulations as per full factorial design to study the effect of two independent variables X1 (amount of span-60), and X2 (amount of cholesterol) on three dependent variable Y1 (percent drug entrapment efficiency), Y2 (percent drug content) and Y3 (percent cumulative drug release) respectively. The relation between the dependent and independent variables was drawn out from the mathematical equation and response surface methodology (RSM). Statistical analysis was performed using ANOVA. Microscopic observation confirmed that all particles were uniform in size and shape. The particle size of niosomes measured by SEM was between 3 µm to 4.5 µm that given the evidence of large uni-lamellar vesicles formed

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by EIM and TFHM. The percent drug entrapment efficiency was found to be highest for formulations MTEIM-8 and MTTFHM-8 with values 97.11% and 95.56% respectively. *In vitro* dissolution studies were carried out in phosphate buffer (pH 6.8) for 8 hours at 100 rpm and maintained at 37 ± 0.5^0C according to USP-II paddle method and absorbance was taken at 226 nm. The probable drug release mechanism may be fickian (class I) diffusion as the correlation coefficient (R^2) best fitted with zero order and release exponent (n) was less than 0.43. The FTIR studies have been done to confirm no interaction along with drug and polymer. *In vitro* and *ex vivo* comparative studies showed that niosomes had controlled the release of drug for a longer period. Finally, it can be concluded that niosomes could be an effective vesicle for delivery of MT with increased bioavailability.

Keywords: Niosomes; metoprolol tartrate; EIM; TFHM; factorial design; bioavailability.

1. INTRODUCTION

In recent years, a major goal for the drug delivery research is renewed towards the development of efficacious drug delivery systems with already existing active ingredients in case of new drug discovery [1]. Many of pharmaceutical therapeutic agents are mostly effective when made available at constant rates or near to absorption sites [2]. Much effort has been going on to develop sophisticated drug delivery systems such as niosomes for oral application. Niosomes are a novel drug delivery system, in which vesicles are microscopic lamellar structures formed on admixture of nonionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. The vesicles are composed of bilayers of nonionic surface active agents and hence the name niosomes. Niosomes can entrap both hydrophilic and lipophilic drugs, either in an aqueous layer or in a vesicular membrane made of lipid material. It can prolong the circulation of entrapped drugs. Because of the presence of nonionic surfactants with the lipid, there is better targeting of drugs to the tumour, liver and brain. It may prove very useful for targeting the drug for treating cancer, parasitic, viral and another microbial disease more effectively [3,4].

Metoprolol tartarate (MT) is a synthetic, cardio-selective β1-adrenoreceptor antagonist widely used in the treatment of essential hypertension and other cardiac disorders. After oral administration, MT is almost completely absorbed (95%) with peak plasma concentrations achieved after 2–3 hours. It undergoes extensive first-pass metabolism by the liver that results in low and variable oral bioavailability (40%–50%). The plasma half-life is approximately 3–4 hours, which needs frequent dosing to maintain the therapeutic blood levels of the drug for a long-term treatment [5,6]. Several attempts have been attained to enhance its bioavailability by avoidance of first-pass hepatic metabolism such as intravenous, transdermal, intranasal, rectal, and Bucco-adhesive drug delivery systems. Other strategies with sustained drug release pattern have been developed to avoid the frequent dosing of MT, including the use of floating tablets, mucoadhesive floating beads, microspheres, niosomes, and proniosomes [7,8].

Drug delivery systems using colloidal particulate carriers have significant advantages over conventional dosage forms as the particles can act as a reservoir for the loaded drug. Niosomes are closed bilayer vesicles formed by self-assembly of nonionic surfactants in aqueous media [9,10]. These structures are analogous to liposomes but have the ability to increase the stability of their entrapped drugs [11]. Due to the flexibility of their structural characteristics (composition, fluidity, and size) and ease of storage and handling, these lipid vesicles can be tailored for delivery of a wide variety of drugs for drug targeting, controlled release, and per-meation enhancement [12].

In the present study, an attempt has been made to prepare and evaluate MT-loaded niosomes suspension. The oral niosomal suspension is expected to enhance the drug bioavailability by avoidance of first-pass hepatic metabolism. In addition, rapid absorption of the drug-loaded liposomes may maintain therapeutic concentrations of the MT for the prolonged time period and, thus, avoiding the frequent dosing of the drug.

2. METHODS AND MATERIALS

2.1 Materials and Instruments

Metoprolol tartrate was Gift sample by Square Pharmaceuticals Ltd. Bangladesh. Span 60,

methanol, propanol-1 and di ethyl ether was purchased from MERCK, Germany. Cholesterol (CHO) was purchased from ALFA Aesar, Great Britain, UK. All other ingredients used throughout the study were of analytical grade. USP Type I, Type II Dissolution Apparatus (VEEGO, India), UV-VIS Spectrophotometer (UV mini-1240) (Shimadzu Corporation, Japan), Rotary Flash Evaporator (YAMATO, Japan), Centrifuge model 400 (Shimadzu Corporation, Japan) etc.

2.2 Niosome Preparation

For the preparation of MT loaded incomes using the central composite design of user-defined factorial design (Design Expert® software-Trial Version 7.1.6, Stat-Ease Inc., MN) was adopted to optimize the formulation parameters and to study the influence of independent formulation variables on dependent variables. Nine experimental runs were designed by selecting two parameters (span-20 and cholesterol amount) at three levels each (low, medium and high) that is shown in Table 1. The amount of drug (50 mg) was kept constant for each batch. Percent Drug Entrapment Efficiency (Y1), Percent Drug Content (Y2) and Percent *in vitro* release (Y3) were selected as dependent variables. The results obtained for each response were fitted to a quadratic polynomial model explained by a nonlinear equation:

$$Y=\beta_{0+}\beta_1X_1+\beta_2X_2+\beta_3X_1X_2+\beta_4X_1^2+\beta_5X_2^2$$

Where Y is the measured response, β_0– β_5 are regression coefficients and X1 and X2 are independent factors. The models were validated by analysis of variance (ANOVA) and multiple correlation coefficient (R^2) tests.

2.2.1 Preparation of metoprolol tartrate niosomes by ether injection method (EIM)

Cholesterol (CHO) and surfactant were dissolved in 8 ml diethyl ether mixed with 2 ml methanol containing a weighed quantity of MT. The resulting solution was slowly injected using a micro syringe at a rate of 1 ml/min into 20 ml of hydrating solution phosphate buffer (pH 6.8). The solution was stirred continuously on a magnetic stirrer and the temperature was maintained at 60-65ºC. As the lipid solution was injected slowly into the aqueous phase, the differences in temperature between phases cause rapid vaporization of ether, resulting in spontaneous vesiculation and formation of niosomes, which is shown in Fig. 1 and Table 2. A 2^3 (two factors, three levels) central composite design was employed to study the effect of independent variable on dependable variables. All the formulations as per experimental design were prepared using similar procedure by addition of various quantities of surfactant and cholesterol.

2.2.2 Preparation of metoprolol tartrate niosomes by thin film hydration method (TFHM)

In this method surfactant (span 60) and cholesterol were dissolved 8 ml diethyl ether. A weighed quantity of drug was dissolved in 2ml methanol. Then the two solutions were mixed together in a round bottom flask. Using the rotary flash evaporator, the organic solvents were removed at room temperature of 20ºc. The flask was rotated at 135 RPM which leaves a thin layer of solid mixture on the wall of the flask that is shown in Fig. 1. The dried film is then rehydrated with 20 ml 6.8 phosphate buffer solution at the temperature of 60-65^{0}c for a specified period of time (about 3 hours) with gentle agitation. Finally, the niosomal dispersion was stabilized by keeping at 2-8^{0}C for 24 hours. All the formulations as per experimental design were prepared using similar procedure by addition of various quantities of surfactant and cholesterol.

2.3 Evaluation of Metoprolol Tartrate Loaded Niosomes

2.3.1 Determination of percentage of drug encapsulated in the niosomes

Entrapment efficiency was measured by measuring the un-entrapped free drug. The free drug was determined by subjecting the niosomal formulation to centrifugation at 4000 rpm for 2 hrs to separate the free drug. After centrifugation, the supernatant was collected. The collected supernatant was analyzed for the drug content spectrophotometrically at 226 nm. The per cent entrapment was determined by following formula:

$$\%\ \text{Drug Entrapment efficiency} = \frac{\text{Amount of Entrapped Drug}}{\text{Total Amount Added}} \text{X } 1oo$$

2.3.2 Drug content

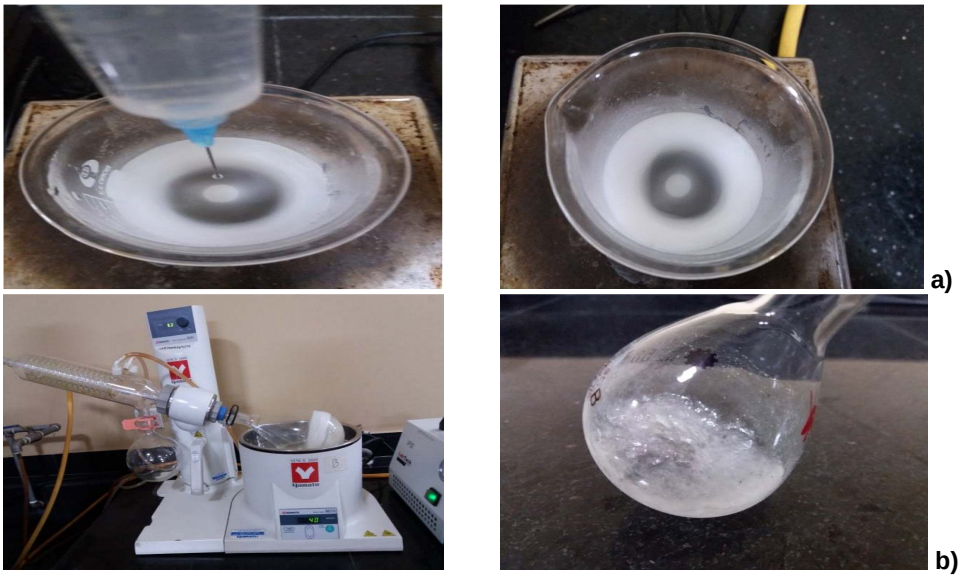
Drug content was determined by disrupting the niosomal formulation by propane-1-ol, diluted suitably using phosphate buffer pH 6.8 (6.805 g/L

potassium phosphate monobasic and ~0.896 g/L sodium hydroxide) and analyzed for the drug content spectrophotometrically at 226 nm. The percentage of drug content was calculated by using the following formula:

$$\% \text{ Drug content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \text{X} \frac{\text{Standard dilution}}{\text{Sample dilution)}} \text{X100}$$

2.3.3 Stability studies

The stability studies of the optimized liposomal formulations were performed at different conditions of temperature and the effect on physical characteristics and drug content was noted. The niosomal dispersions were kept in the air tight containers and stored at refrigeration temperature (2-8^{o}C) and at room temperature



a)

b)

Fig. 1. Preparation of Niosomes by a) Ether Injection Method (EIM) and b) Thin Film Hydration Method (TFHM) Respectively

Table 1. Independent variables and their levels in experimental design

Independent variables	Levels (Actual Coded)		
	Low (-1)	Medium (0)	High (+1)
X1: Span-60 (mg)	50	100	150
X2: Cholesterol (mg)	50	100	150
Dependent variables		**Goals**	
Y1: Drug Entrapment Efficiency (%)		Maximize	
Y2: Drug Content (%)		Maximize	
Y3: CDR at 8 hrs (%)		Minimize	

Table 2. Design layout of experiments as per user defined factorial design

		Coded Value		Actual Value (mg)	
Run	**Drug (mg)**	**Span 60**	**Cholesterol**	**Span 60**	**Cholesterol**
R1	50	-1	0	50	100
R2	50	-1	+1	50	150
R3	50	0	+1	100	150
R4	50	-1	-1	50	50
R5	50	-1	0	50	100
R6	50	0	0	100	100
R7	50	0	-1	100	50
R8	50	+1	+1	150	150
R9	50	+1	-1	150	50

(30 ± 2^{o}C) for 21 days and the 1.0 ml samples were withdrawn on different days (7, 14 and 21). The stability of formulation was analyzed by measuring entrapment efficiency and drug content.

2.3.4 Morphological characterization of niosomes

The vesicle formation by the particular procedure was confirmed by optical microscopy in 45x resolution. The niosomal suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film and ether injection of niosome suspension observed for the formation of vesicles. The microphotography of the niosomes also obtained from the microscope by using a digital camera (Fig. 2). The detailed surface characteristic of the selected MT niosomes formulation was observed using a scanning electron microscope.

2.3.5 Particle size by scanning electron microscopy (SEM)

Vesicle size of selected ribosomal dispersion was determined by an optical microscope and vesicle size, shape and surface property of the selected formula was studied using scanning electron microscope.

2.3.6 Drug-excipient compatibility study by FTIR spectroscopy

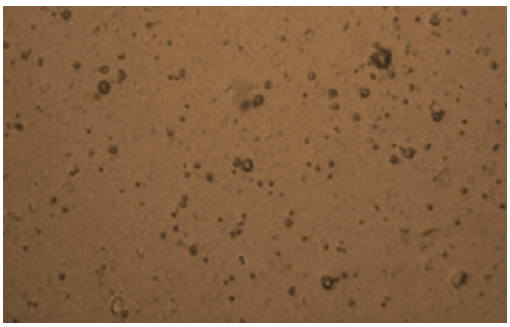
Drug-excipient compatibility studies were done in order to evaluate any interaction between drug and polymers used in the preparation of niosomes. FTIR spectroscopy was carried out to check the compatibility between MT and surfactants used.

2.4 *Ex vivo* Permeability Study Using Chicken Intestinal Sac

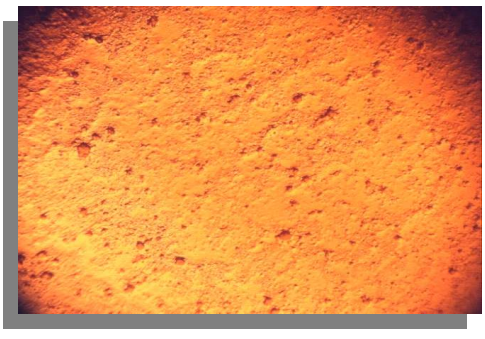
Ex vivo permeability study was carried out by using chicken intestinal sacs. Phosphate buffer (6.8) was used as dissolution media. Preparation (1.5 ml niosomal suspension) was used. Dissolution studies were conducted in a dissolution apparatus using USP II paddle method. For isolation of everted intestine, the chicken was bought from the local market and was slaughtered. The lumen was carefully cleared from mucus by rinsing with a phosphate buffer solution (pH 6.8) (Krebs–Ringer solution).Total nine intestinal segment of six cm length were removed and transferred to oxygenated Krebs-Ringer solution. 1.5 millilitres of niosome suspension was placed in the sac which was then sealed at both ends. The sac was dipped into the receptor compartment containing the dissolution medium, 900 mL of phosphate buffer (pH 6.8), was stirred continuously at 100 rpm and maintained at 37^{0}C. 10 ml of the sample was withdrawn at predetermine intervals from each basket, filtered with 0.45 µ filter paper and media was replenished by fresh medium. The permeability study was checked for eight hours. Fig. 3a represents the steps of *ex vivo* permeability study of metoprolol tartrate loaded niosomes using chicken intestinal sacs.

2.5 *In vitro* Permeability Study Using Cellulose Dialysis Tubing

In vitro permeability study was done using cellulose dialysis membrane (Specrtapor, USA) in USP II paddle method. Dialysis membrane was cut into nine (9) cm in length and soaked them in 500 ml distilled water at room



a)



b)

Fig. 2. Particle Size Observation by Optical Microscopy of Metoprolol Tartrate Niosomes a) MTEIM and b) MTTFHM respectively

Chicken Intestinal Membrane

a)

Dialysis Sac Put into Dissolution Vessel

Cellulose Dialysis Membrane

b)

Dialysis Sac Put in to Dissolution Vessel

Fig. 3. Schematically Presenting Permeability Study of Metoprolol Loaded Niosomes a) *ex vivo* **Using Chicken Intestinal Sacs and b)** *in vitro* **Using Cellulose Dialysis Tubing Membrane respectively**

temperature for 30 minutes to remove the sodium azide preserving agent. Then the membrane was rinsed thoroughly in distilled water. 1.5 ml of niosome suspension was placed in the membrane which was then sealed at both ends. The membrane was dipped into the receptor compartment containing the dissolution medium, 900 mL of phosphate buffer (pH 6.8), was stirred continuously at 100 rpm and maintained at 37^0C. 10 ml of the sample was withdrawn at predetermine intervals from each basket, filtered with 0.45 µ filter paper and media was replenished by fresh medium. Absorbance was taken by using UV spectrophotometer at 226 nm. The permeability study was checked for eight hours. Fig. 3b shows the steps of *in vitro* permeability study of MT loaded niosomes using cellulose dialysis membrane.

2.6 Interpretation of Dissolution Profile of Niosomes

2.6.1 Interpretation of dissolution profile

Absorbance values obtained from the dissolution studies were converted into per cent release of drug from the formulations of niosomes. This is done by comparing the absorbance values with the standard curve.

2.6.2 Release kinetics

Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism of drug release from the noise. The kinetic models used were zero order first-order, Higuchi and Korsmeyer-Peppas to ascertain the kinetic modelling of drug release.

2.7 Successive Fractional Dissolution Time

To characterize the drug release rate in different experimental conditions, T25%, T50% (mean dissolution time) and T80% were calculated from dissolution data according to the following equations

- T25% = (0.25/k)1/n
- T50% = (0.5/k)1/n
- T80% = (0.8/k)1/n

Mean Dissolution Time can also be calculated by the following equation [13].

- MDT = (n/n+1). $K^{-1/n}$

Mean dissolution time (MDT) value is used to characterize the drug release rate from the niosomes and the retarding efficiency of the

surfactant and cholesterol. A higher value of MDT indicates a higher drug retaining the ability of the surfactant and vice-versa.

2.8 Statistical Analysis by Factorial Design

Statistical analysis was done using ANOVA. Nine experimental runs were designed (Design Expert® software-Trial Version 7.1.6, Stat-Ease Inc., MN) by selecting two parameters (span-20 and cholesterol amount) at three levels each (low, medium and high). The regression parameters of the developed model and graphical interpretation for each response with statistical significance ($p<0.05$) were calculated by using design expert.

2.9 Optimization Using the Desirability Function

To optimize multiple responses, they should be highly correlated with each other. In the present study, all three responses were simultaneously optimized by a desirability function that uses the numerical optimization method in the design-expert software. Recently, the desirability function approach was reported in several articles for the optimization of multiple responses. Any response that falls outside the desired limit is considered completely unacceptable. For the response to be maximized, the desirability function can be defined as:

$$d_{i,\min} = \frac{Y_{\max} - Y_i}{Y_{\max} - Y_{\min}}$$

Where, *di*, max is the individual desirability of the response to be maximized, *Yi* is the experimental result, and *Y*min and *Y*max represent the minimum and maximum possible values. If *Yi* is equal to or less than *Y* min, then *di*, max = 0; and if *Yi* is higher or equal to *Y*max, then *di*,max = 1. In order for the response to be minimized the desirability function is defined as:

$$d_{i,\max} = \frac{Y_i - Y_{\min}}{Y_{\max} - Y_{\min}}$$

Where if *Yi* is higher than or greater than *Y*max, then *di*, min = 0; and if *Yi* is less than or below the minimum, then *di*, min = 1. After obtaining the individual desirability values for each response, the results are usually combined as a geometric mean to give a global desirable value (*D*), which is explained by equation:

$$D = (d_1 \times d_2 \times d_3 \times d_4 \times \ldots\ldots\ldots \times d_n)^{1/n} = \left(\prod_{i=1}^{n} d_i\right)^{1/n}$$

Where, *n* specifies the number of responses being optimized. According to the simultaneously assigned goals for all responses, the design-expert software determines the maximum desirability value by an extensive grid search over the domain [14].

3. RESULTS AND DISCUSSION

3.1 Analysis of Percent Drug Entrapment Efficiency (DEE) and Percent Drug Content (DC) of Metoprolol Tartrate Loaded Niosomes Prepared by EIM and TFHM

Different ratios formulated niosomes were analyzed for their percent drug entrapment efficiency (DEE) and percent drug content (DC). Results of the study were illustrated in the Fig. 4.

MT loaded niosomes prepared using span 60 and cholesterol by EIM and TFHM were shown in Fig. 3. From the figure it can be said that percent drug entrapment efficiency of different formulations were in range of 70.16% to 97.11% whereas drug content ranging from 89.33% to 94.66% for EIM and percent drug entrapment efficiency of different formulations formed by thin film hydration method were in range of 79.34% to 95.56% whereas drug content ranging from 75.94% to 99.50%. The entrapment efficiency was found to be higher with the formulations which have high cholesterol and surfactant ratio to provide a high entrapment efficiency of MT. Increase in the concentration of the surfactant leads to enhancement in the encapsulation efficiency and decrease in the leakage of the drug which might be due to the high fluidity of the vesicles but it depends upon the cholesterol amount. Minimum cholesterol content of formulations was found to cause low entrapment efficiency. This might be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment.

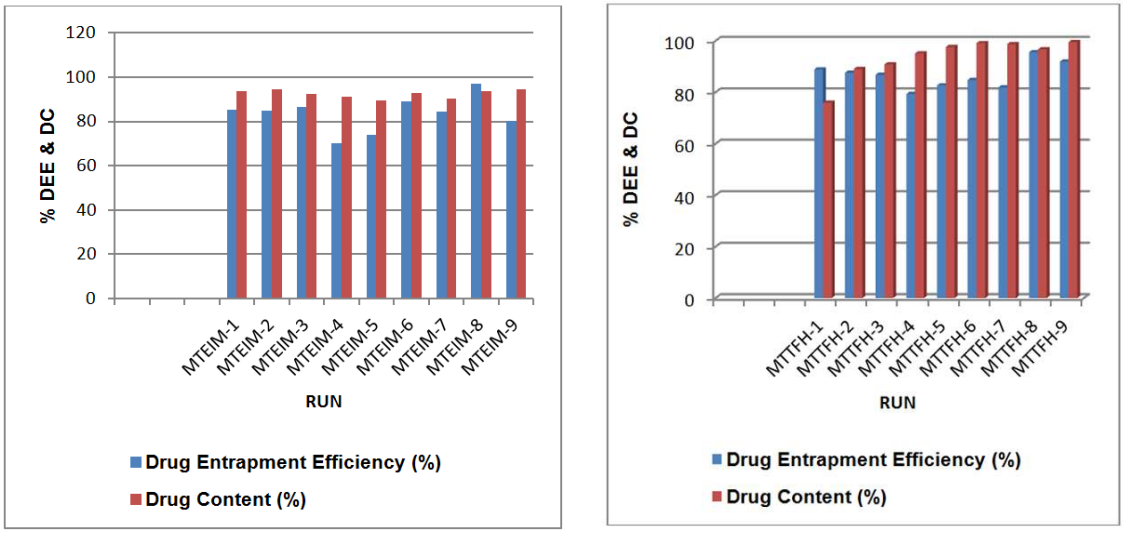


Fig. 4. Percent Drug Entrapment Efficiency (DEE) and Percent Drug Content (DC) of metoprolol Tartrate Niosomes a) MTEIM-1 to MTEIM-9 and b) MTTFH-1 to MTTFH-9 Respectively

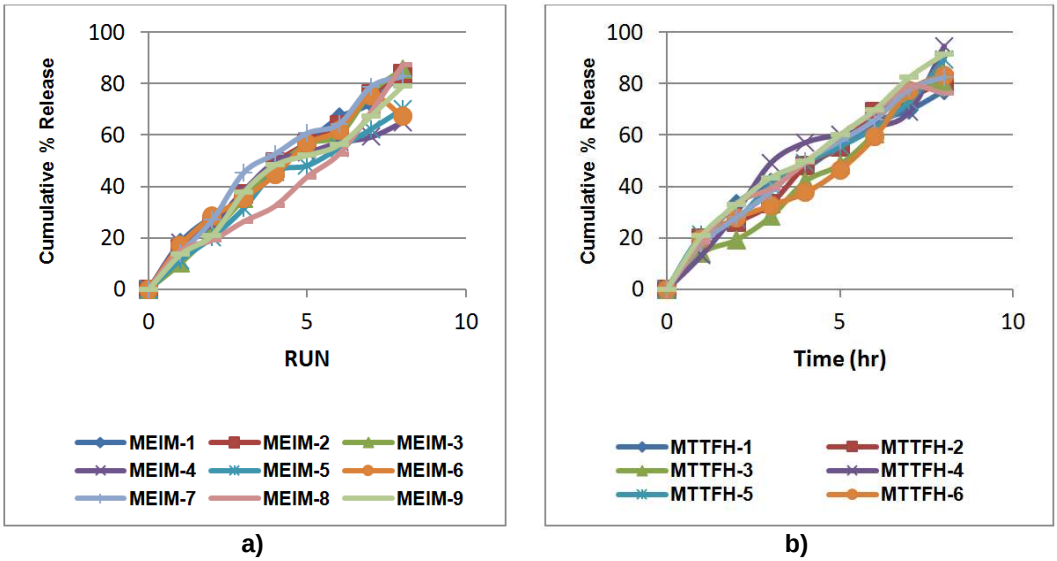


Fig. 5. Zero-order Release Kinetic Plot of Metoprolol Tartrate Loaded Niosomes Prepared by a) EIM and b) TFHM Respectively

3.2 *In vitro* Drug Release Studies of Metoprolol Tartrate Loaded Niosomes

In vitro release profile of MT loaded niosomes given in Fig. 5 depicts the release kinetics plot of MT loaded niosomes.

From the Fig. 5, it has been found that the percentage release of drug, however, increased with time for formulations MTEIM1 to MTEIM9 and MTTFHM1 to MTTFHM9. After 8 hours dissolution, the maximum drug release was found 88.90 % and 89.45% whereas the minimum release was 65.12% and 76.20% for EIM and TFHM respectively. It was observed that amount of surfactant and cholesterol affected the drug release. The increased in a surfactant (span 60) normally decreased in drug release that was seen in the formulations but a negative effect

also observed in the formulations. Hence increase in the surfactant increased the drug release. The release was more controlled by increasing the cholesterol level. From the per cent release of drugs, it was seen that by increasing the cholesterol ratio the drug release decreased because cholesterol acted as a retardant barrier.

To find out the probable drug release mechanism interprets the release rate constants and R^2 values for different release kinetics of MT loaded niosomes. All the formulations were best fitted with zero-order models as shown in Table 3. The data obtained were also put in zero-order models in order to find out n value, which describes the drug release mechanism. The n value of niosomes of the different drug to polymer ratio was ranged less than 0.43, indicating that the mechanism of the drug release was fickian diffusion controlled.

3.3 *Ex vivo* Drug Release Studies of Metoprolol Tartrate Loaded Niosomes formed by a) EIM and b) TFHM Respectively

Ex vivo Release Profile of MT loaded niosomes formed by a) EIM and b) TFHM is given in Fig. 5.

In permeability studies, the Fig. 6 indicated that the percentage drug released however increased with time. In case of ether injection method, the highest cumulative per cent release was 92.11% for formulation MTEIM-4 and the lowest cumulative per cent release was 77.09% for MTEIM-8. It was observed that amount of surfactant and cholesterol affected the drug release profile. In case of thin film hydration method, the highest cumulative percentage release was 97.48% for MTFHM-4 and the lowest cumulative per cent release was 79.67% for MTFH-8. However, from *ex vivo* permeability studies it is confirmed that the drug release decreased by increasing the amount of cholesterol and surfactant.

3.4 Comparative Study of MT Loaded Niosomes

In vitro and ex vivo, comparative release studies have shown in Fig. 7 for the pure drug, marked product and formulated niosomes (MTEIM-4 and MTTFHM-4). At the end of 8 hours, all MT loaded niosomes showed higher diffusion against pure drugs and marketed products. *Ex vivo* permeability study through chicken intestinal sacs is one of the essential parts in the prediction of oral bioavailability. Besides, it can be said from the figure that MT loaded niosomes by thin film hydration method (TFHM) has shown better-controlled release over the period of time than ether injection method (EIM).

Table 3. Interpretation of release rate constants and R^2 values for different release kinetics of different niosomal formulations of metoprolol tartrate

Formulation code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	n	R^2
MEI-1	9.376	0.987	-0.085	0.961	29.187	0.983	0.321	0.231
MEI-2	9.686	0.998	-0.080	0.968	30.904	0.945	0.378	0.195
MEI-3	8.419	0.996	-0.888	0.978	31.104	0.958	0.299	0.250
MEI-4	10.621	0.992	-0.088	0.986	28.351	0.965	0.286	0.178
MEI-5	7.708	0.990	-0.085	0.945	33.786	0.980	0.450	0.248
MEI-6	8.653	0.991	-0.076	0.989	24.689	0.950	0.364	0.196
MEI-7	10.182	0.992	-0.093	0.940	31.402	0.970	0.375	0.245
MEI-8	10.241	0.979	-0.065	0.930	27.691	0.978	0.341	0.250
MEI-9	9.355	0.998	-0.082	0.987	24.871	0.997	0.310	0.143
MTTFH-1	15.122	0.989	-0.119	0.987	29.089	0.987	0.348	0.234
MTTFH-2	9.389	0.996	-0.143	0.994	30.782	0.991	0.302	0.198
MTTFH-3	10.104	0.979	-0.089	0.917	30.636	0.909	0.227	0.176
MTTFH-4	11.987	0.993	-0.109	0.973	32.278	0.989	0.331	0.256
MTTFH-5	9.688	0.996	-0.090	0.989	29.345	0.961	0.312	0.321
MTTFH-6	10.345	0.995	-0.087	0.992	29.989	0.958	0.470	0.250
MTTFH-7	8.7711	0.989	-0.165	0.970	26.905	0.980	0.307	0.184
MTTFH-8	9.206	0.999	-0.050	0.960	27.379	0.984	0.274	0.143
MTTFH-9	10.104	0.997	-0.089	0.997	27.203	0.994	0.309	0.165

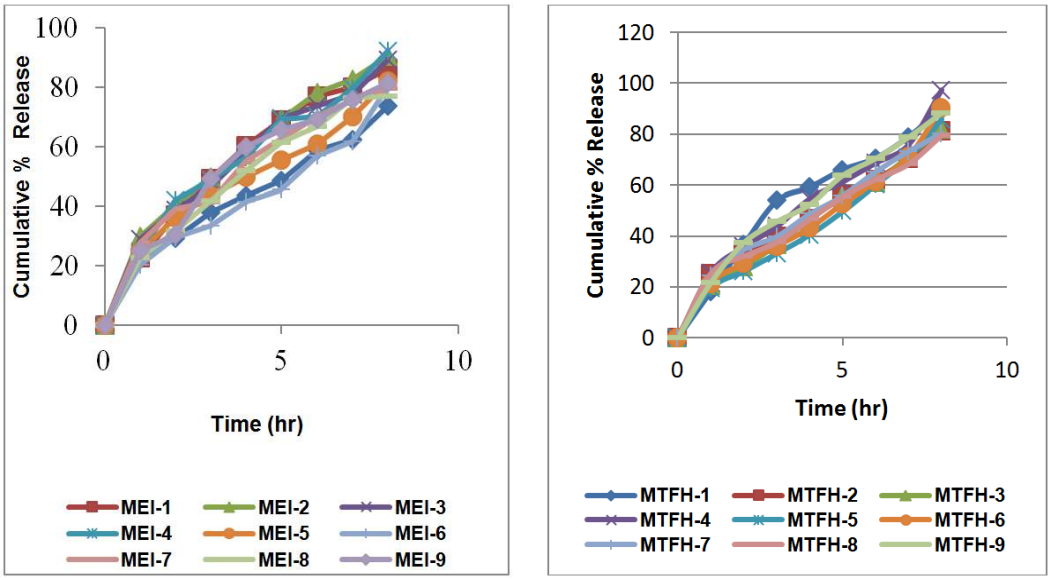


Fig. 6. *Ex vivo* Release Plot of Metoprolol Tartrate Loaded Niosomes prepared by a) EIM and b) TFHM respectively

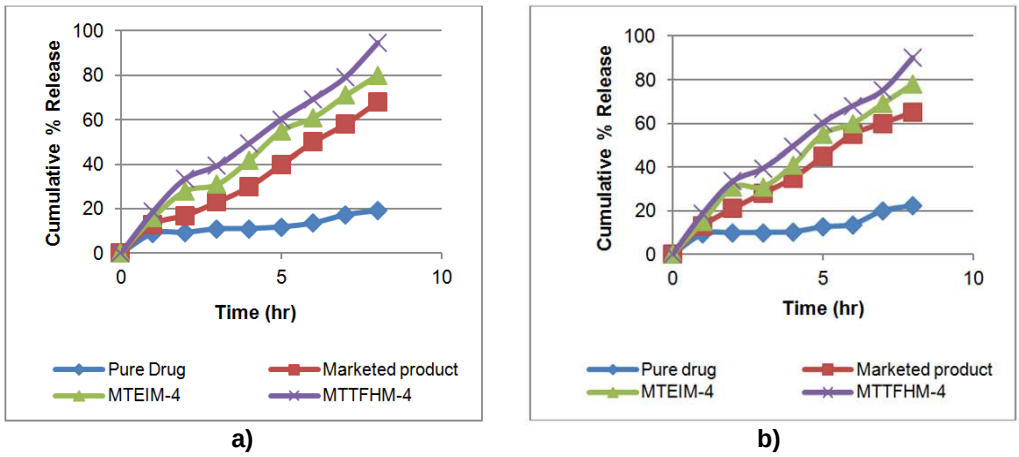


Fig. 7. Comparative Study of Metoprolol Tartrate Loaded Niosomes a) *in vitro* Release Plot and b) *ex vivo* Release Plot Respectively

3.5 Successive Fractional Dissolution Time

Successive fractional dissolution times (hr) of MT loaded niosomes of different formulations are shown in Fig. 8. To characterize the drug release rate in different experimental conditions they were calculated from dissolution data. The overall results of MDT value are showing that if the amount of surfactant and cholesterol is increased the retarding affinity of formulations also increases but it happens only for a certain level. After an optimum level, increasing in the amount of surfactant and cholesterol results in decreased drug retarding affinity because after the optimum level cholesterol starts to break the bilayer of the vesicle which has to be controlled by the amount of surfactant.

3.6 Stability Studies

To obtain stability data of different niosomal formulations, they were kept under the different condition for several days that are given in

Table 4. The intermediate stability study for MTEIM-6 and MTTFHM-5 was performed for 21 days according to the ICH guidelines. Drug entrapment was fixed as a physical parameter for stability testing and stability studies of selected formulation MTEIM-6 and MTTFH-5 showed that negligible changes in entrapment efficiency. This revealed that the formulations stabled on storage at 4±2ºC and 30±2℃ RH.

3.7 Analysis of Particle size or Vesicle Formation

The particle size of niosomal formulation at different ratio of cholesterol and surfactant was shown in Table 5. The mean particle size of the niosomal formulation was found to be in the range of 3.3 μm to 4.5 μm. It was clearly depicted from the figure that particle size of niosomal formulations was increased on increasing the cholesterol (CHO) content. CHO content provides strength to the nonpolar tail of nonionic surfactant. At low CHO content, it is to be expected that the CHO and nonionic surfactant are in close packing with increasing curvature and reducing size. As the CHO content increases, it would reduce the content of surfactants and also increased the hydrophobicity of bilayer membrane thus increasing vesicles radius in a way to establish more thermodynamic stable form. Rigid structure of bilayer membrane due to cholesterol content also provides resistance to reduce size due to sonication and results in vesicles with bigger size. In addition, it can be said from the table that MT loaded niosomes by thin film hydration method (TFHM) has shown larger vesicles or particle size than ether injection method (EIM).

3.8 Fourier Transform Infrared Spectroscopy (FTIR) Study of Metoprolol Tartrate Loaded Niosomes

FTIR study has done to examine drug-polymer interaction. To probe this effect FTIR was performed on a) Pure drug (Metoprolol Tartrate) b) Span-60 c) Cholesterol d) Metoprolol tartrate loaded liposomes prepared by EIM e) Metoprolol tartrate loaded niosomes prepared by TFHM shown in Fig. 9. FTIR spectrum of MT shown in Fig. 9a revealed that the functional group C=O appeared at a wave number of 1604.17 and 1512.19 cm-1, whereas -CH3 appeared at a wave number of 1332.61 and 1306.81 cm-1. -NH2 appeared at wave number 1264.09 and 1111.01 cm-1. According to FTIR spectrum, the functional groups C=O, -CH3 and -NH2 appears at 1675 – 1500, 1475 – 1300 and 1250 -1050 cm-1 region. It means that MT meets the requirements. FTIR spectrum of formulation MEI-3 and TFH-2 has shown the peak within the limit. There was no appearance of any characteristics peaks that were shown in FTIR spectrum of MT loaded niosomal formulations MEI-3 and TFH-2. This confirmed that there was no interaction between the drug and excipients used in the preparation of MT loaded niosomes.

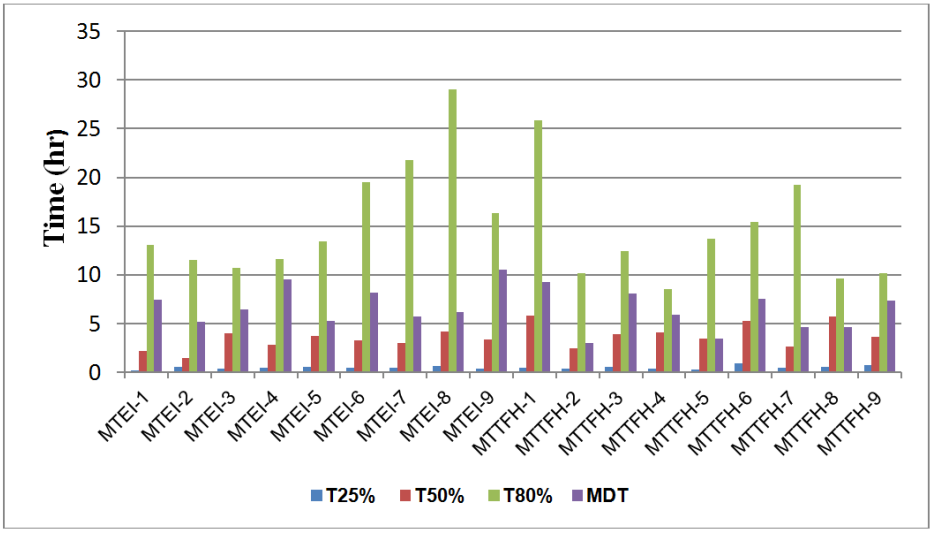


Fig. 8. Successive Fractional Dissolution Time of Metoprolol Tartrate Loaded Niosomes by EIM and TFHM Respectively

Table 4. Stability studies of metoprolol tartrate loaded niosomes

RUN	MTEI-6		MTTFH-5	
Duration	Refrigeration Temp. (4±2°C)	Room Temp. (30±2°C)	Refrigeration Temp. (4±2°C)	Room Temp. (30±2°C)
7 days	89.22	89.20	82.67	82.62
14 days	89.09	89.03	82.59	82.45
21 days	88.97	88.95	82.10	82.19

Table 5. Mean Particle Size (µm)

Run	Mean Particle Size (µm)	Run	Mean Particle Size (µm)
MEI-1	3.55±0.04	MTTFH-1	3.65±0.07
MEI-2	3.67±0.06	MTTFH-2	4.17±0.08
MEI-3	3.84±0.10	MTTFH-3	4.24±0.9
MEI-4	3.31±0.12	MTTFH-4	3.41±0.15
MEI-5	3.44±0.19	MTTFH-5	3.54±0.13
MEI-6	3.73±0.11	MTTFH-6	3.83±0.14
MEI-7	3.33±0.13	MTTFH-7	3.43±0.16
MEI-8	4.11±0.25	MTTFH-8	4.50±0.18
MEI-9	3.61±0.07	MTTFH-9	3.74±0.11

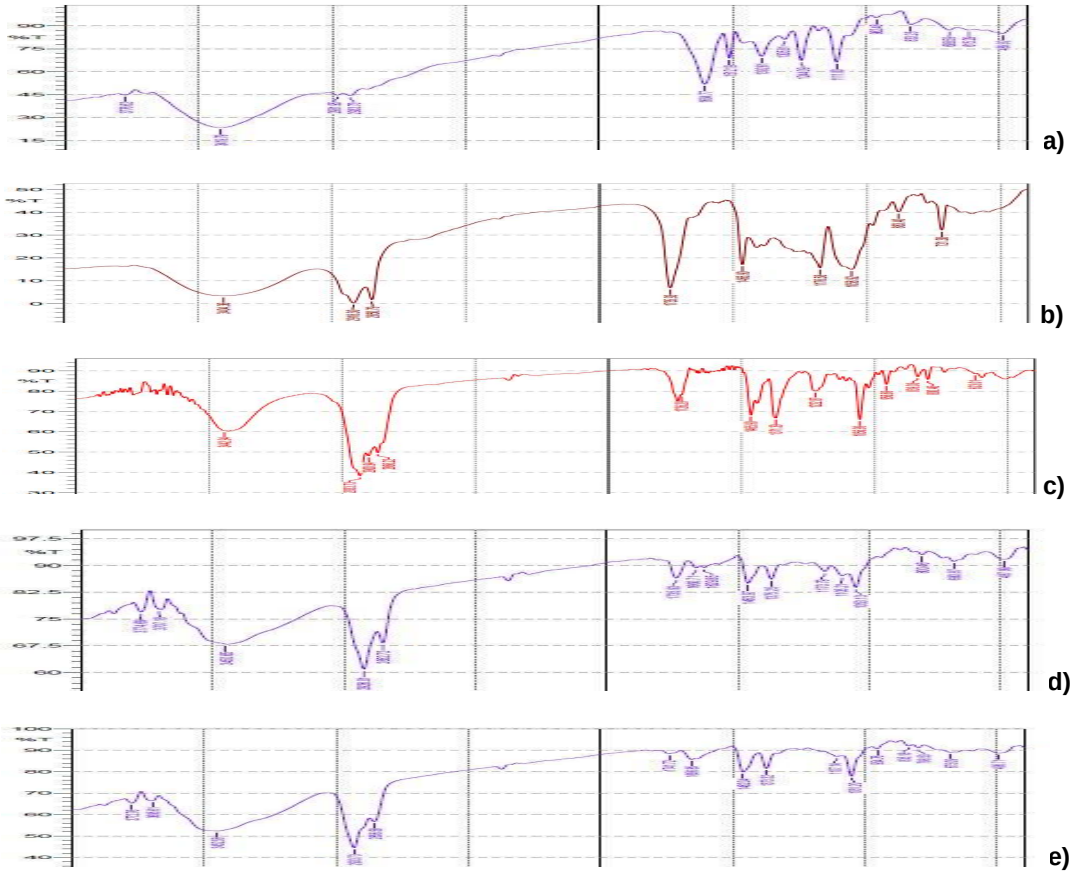


Fig. 9. FTIR of a) Metoprolol Tartrate b) Span 60 c) Cholesterol d) Formulation MTEIM-3 and e) Formulation TFHM-2 respectively

3.9 Scanning Electron Microscopy of Metoprolol Tartrate Loaded Niosomes

The surface morphology of the niosomes was investigated by SEM. The images of (a) MTEIM-3 and (b) MTTFH-3 were shown in Fig. 10 to see the morphological changes that occurred due to formulation variation. Surface morphology of formulation MTEIM-3 and MTTFH-3 indicates that niosomal particles were appeared as discrete and round in shape with irregular surface due to the presence of entrapped drug. Vesicular properties of these drug carriers which formed from double layers. SEM showed the morphology of the lipids and the arrangement of the lamellar structure the encore the drug molecules.

3.10 Full Factorial Statistical Analysis

3.10.1 ANOVA tests of the quadratic model and regression analysis for the responses

The regression parameters of the developed model and graphical interpretation for each response with statistical significance were calculated design expert using full factorial design shown in Table 6 and Table 7. The relationship between the experimental variables and responses were evaluated by generating response surface plots. In the ANOVA test, the p values of the model for responses Y1, Y2 and Y3 were 0.0345, 0.0289 and 0.0185 for MTEIM and 0.0125, 0.0375 and 0.0481 for MTFDM respectively. Thus, from the p values for this model it can be concluded that all the responses (Y1, Y2 and Y3) fitted the quadratic model well ($p < 0.05$). Significance probability values (Probability > F) less than 0.05 implies that the model is significant. Moreover, in the 'lack of fit' test, which is another good statistical parameter for checking the better fitness of the model, all the responses fitted in the quadratic model by showing a non-significant lack of fit ($p>0.1$). In this study, the R^2 values for the responses Y1, Y2, and Y3 were 91.75 %, 95.18%, and 90.20% for MTEIM and 91.81%, 95.72%, and 92.36% for MTFHM, respectively.

Mathematical relationship using multiple linear regressions

- **Final Equation In Terms of Coded factors**
  % Drug Entrapment Efficiency (Y1) =+88.50+5.80 * A+5.58 * B+0.57 * A * B-2.86 * A^2-2.76* B^2
- **Final Equation in Terms of Actual Factors**
  % Drug Entrapment Efficiency (Y1) =+45.53700+0.32197* X1+0.30954 * X2-2.28000E-004 * X1 * X2-1.14340E-003 * X12-1.10320E-003 * X22
- **Final Equation in Terms of Coded Factors:**
  % Drug Release (Y3) =+85.01+8.75* A-0.040* B-2.32* A * B+6.27* A^2-3.22 * B^2
- **Final Equation in Terms of Actual Factors:**
  % Drug Release (Y3) =+71.84800-0.19502*X1+0.29716*X2-1.45000E-004 * X1 * X2
  +1.72640E-003 * $X1^2$-1.28680E-003* X

Table 6. ANOVA tests of the quadratic model and regression analysis for the responses

Method	Response	R^2	Model	Probability > F	Comment
MTEIM	Y1	0.9175	Quadratic	0.0345	Significant
	Y2	0.9518		0.0289	
	Y3	0.9020		0.0185	
	Lack of Fit			**0.48**	Not Significant
MTFHM	Y1	0.9181	Quadratic	0.0125	Significant
	Y2	0.9572		0.0375	
	Y3	0.9236		0.0481	
	Lack of Fit			**0.46**	Not Significant

Table 7. Coefficient estimation for response

Method	Response	Intercept	A-X1	B-X2	AB	A2	B2
MTEIM	Y1	88.50	5.80	5.59	0.57	-2.86	-2.76
MTFHM	Y3	85.01	6.79	1.27	-0.36	4.32	-3.22

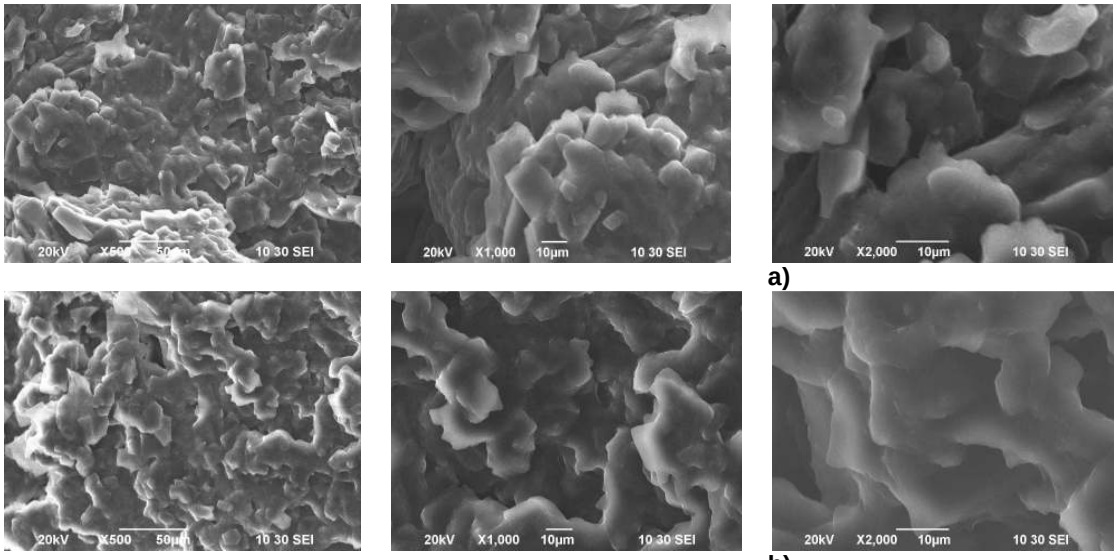
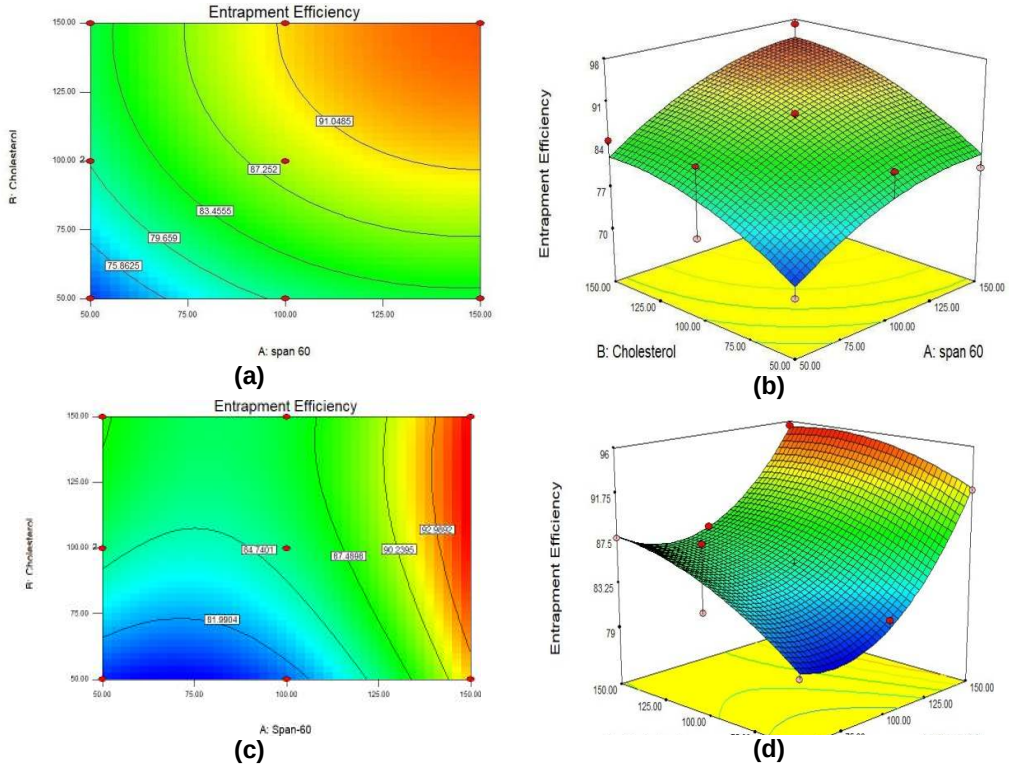


Fig. 10. Scanning Electron Microscopy (SEM) of Formulations a) MTEIM-3 and b) MTTFH-3 at different Magnification respectively

Table 8. Calculated values for optimized solution

Method	X1	X2	Y1	Y2	Y3	Desirability
TFHM	150	150	95.38	96.09	93.79	0.961
EIM	150	150	94.84	93.99	77.90	0.917



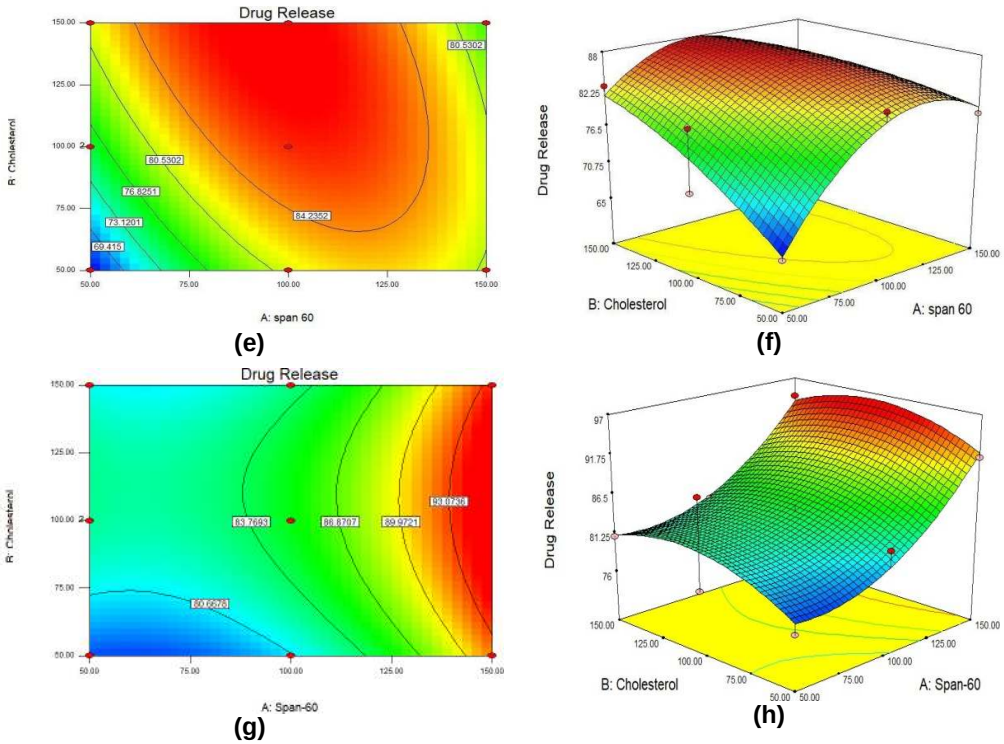


Fig. 11. Contour Plot Showing the Effects of X1 and X2 on the % of Drug Entrapment Efficiency (a, c), and % Drug release (e, g) and Response Surface Showing the Effects of X1 and X2 on the % of Drug Entrapment Efficiency (b, d), and % Drug Release (f, h) for MTEIM and MTFHM Respectively

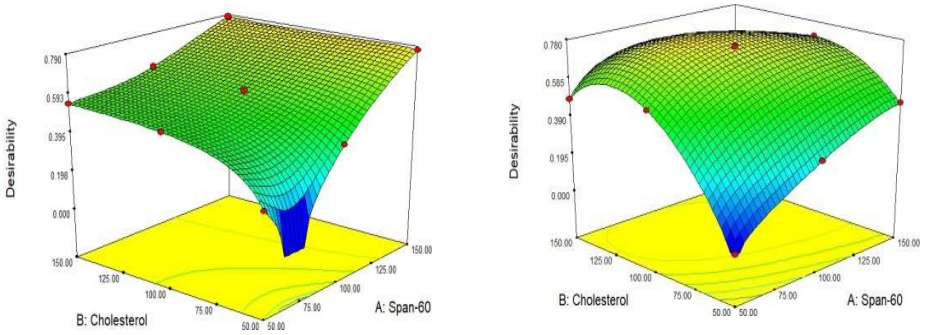


Fig. 12. Response surface Plot Showing Overall Desirability (D) as a Function of X1 and X2 for a) TFHM and b) EIM respectively

3.10.2 Response surface and contour plot analysis

Three-dimensional response surface plots and two-dimensional contour plots of the responses across the selected factors were constructed to further elucidate the relationship between the independent and dependent variables, as shown in Fig. 11. These types of plots are very useful for studying the interaction effects between two factors and for understanding how the effect of one factor will be influenced by the change in the level of another factor. As these types of plots can only express two independent variables at a time against the response, one independent variable must always be fixed.

3.10.3 Optimization using the desirability function of TFHM and EIM

After studying the effects of the dependent and independent variables on the responses, the independent variables were simultaneously optimized for all three responses by using the desirability function. Responses Y1, Y2, and Y3 were transformed into individual desirability shown in Table 8. Constraints were set against all of the responses. Among the responses, Y1 and Y2 were set to be maximized and Y3 were set to be minimized. Equal weight and importance were given to all of the responses. Finally, the global desirability value was calculated by combining the individual desirability function as the geometric mean by an extensive grid search and feasibility search over the domain by the Design-Expert software (Stat-Ease Inc.). Fig. 12 shows the response surface plot for the desirability function holding the variable X1, X2.

4. CONCLUSION

The present study was conducted to design MT loaded controlled release niosomes by ether injection and thin film hydration method. Ether injection method and thin film hydration method are potentially scalable methods for producing niosomes for delivery of hydrophobic or amphiphilic drugs. *In vitro* dissolution study showed the controlled release of drugs from the niosomes for 8 hours. From the *in vitro* dissolution data it has been established that the drug dissolution profile could be sustained by increasing the amount of surfactant and cholesterol in the formulations and where both the surfactant and span-60 are high ensured the better controlled release. Scanning electron microscopy showed uniform size of niosomes. FTIR data showed absence of any new functional group and any other interaction in between drugs and surfactant. In addition formulated niosomes can be chosen for *ex vivo* study by chicken intestine. Niosomal formulations containing MT were successfully optimized by employing statistical tool ANOVA and response surface methodology (RSM). The results suggest that the RSM using factorial design could be a suitable approach for understanding formulation variables and for optimizing the formulation efficiently. The results further reveal that surfactant and cholesterol and its concentration can modify all the evaluation parameters significantly. Moreover, it can be said from the results that thin film hydration method (TFHM) has shown better results in terms of all parameter than ether injection method (EIM). So, MT loaded niosomal drug delivery system might be a potentially controlled drug delivery system for the treatment of hypertension with enhanced bioavailability and patient compliance.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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