In vitro and *Ex vivo* Analysis of Pitavastatin-Loaded Liposomes for the Treatment of Hyperlipidemia

Salma Delwar[1], Swarnali Islam[1], Md. Elias Al-Mamun[2], Nasrin Akter[1] and Irin Dewan[1]

[1]Department of Pharmacy, University of Asia Pacific, 74/A Green Road, Farmgate, Dhaka-1205, Bangladesh
[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka Dhaka-1000, Bangladesh

(Received: October 2, 2022; Accepted: January 12, 2023; Published (web): January 24, 2023)

ABSTRACT: Pitavastatin is a BCS class II drug with low solubility and high permeability. So, the purpose of the study was to develop and characterize pitavastatin-loaded liposomes to achieve sustained release of the drug by increasing the solubility as well as bioavailability in treating hyperlipidemia. All formulations were prepared using the ether injection method (EIM) to determine the effect of three independent factors lecithin, cholesterol, and chitosan/PEG-6000 on two dependent variables of percent drug entrapment efficiency (% DEE) and percent cumulative drug release (% CDR) at low (-1) and high (+1) levels, respectively. The prepared liposomes were analyzed using various techniques, including percent DEE, percent CDR, FTIR, S0EM, and zeta potential. After 8 hours of dissolution, the lowest and highest drug releases were 78.01 and 88.70 percent, 68.12 and 80.19 percent, and 66.22 and 78.91 percent, for FCH-4 and FCH-2, FLE-1 and FLE-3, and FPEG-4 and FPEG-3, respectively. Additionally, permeability experiments were conducted by *in vitro* and *ex vivo* methods. The particle size of liposomes was between 3.51 µm to 4.19 µm that gave evidence of the formation of uni-lamellar vesicles. The release kinetics were investigated using a variety of mathematical models. SEM and FTIR analyses indicated that liposomes have a spherical and smooth surface with no interaction between medications and excipients. Comparative *in vitro* and *ex vivo* studies demonstrated that pitavastatin calcium-loaded liposomes may be viable for patients with hyperlipidemia due to their enhanced solubility and bioavailability.

Key words: Pitavastatin, Liposome, Lecithin, Chitosan, Factorial design.

INTRODUCTION

The research on drug delivery is changing from micro to nano-particulate structures. Nanotechnology is now emerging as a new field of medicine that could yield important therapeutic advantages. The development of nano-delivery devices that can carry drugs precisely and safely to the intended site of action is among the most difficult challenges for researchers studying pharmaceutical formulations. The most common nano-delivery systems are nano-emulsions, polymeric or lipid nanoparticles, and liposomes.[1] Encapsulation of drugs in liposomes has become a new method that allows for precise and controlled delivery. It can improve bioavailability, decrease the toxic effects of drugs, and improve patients' acceptance. Liposomes may deliver the drug to the targeted tissues and reduce the dispersal of drugs to tissues that are not targeted which could lead to more efficient utilization of the drug. Liposomes are small spherical vesicle that is made of a lipid bilayer that has the capability to encapsulate both lipophilic and lipophobic substances.[2]

Pitavastatin is a statin medication that decreases cholesterol levels. Statins work by lowering abnormal cholesterol and lipid levels in the blood and thus lower the risk of cardiovascular diseases. They work by inhibiting the enzyme hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, which converts

Correspondence to: Irin Dewan
E-mail: irin_d@uap-bd.edu

Dhaka Univ. J. Pharm. Sci. **22**(1): 43-54, 2023 (June)
DOI: https://doi.org/10.3329/dujps.v22i1.64145

HMG-CoA to mevalonic acid.[3] It lowers serum cholesterol and LDL levels, lowering the risk of atherosclerosis and associated consequences such as myocardial ischemia and stroke. It has a 50% bioavailability due to its limited solubility. This study aims to increase pitavastatin's solubility and bioavailability by avoiding hepatic metabolism in the first pass. The fast absorption of drug-loaded liposomes may also help to maintain therapeutic pitavastatin concentrations.[4]

Phosphatidylcholine is the most abundant phospholipid in mammalian membranes. The head group contains a quaternary amine (positive charge) and phosphate (negative charge). Chitin is a chitosan polysaccharide. Chitin is the world's second-highest density polysaccharide after cellulose. Chitosan is biocompatible, biodegradable, and harmless, making it excellent for medical and pharmaceutical applications. Organic polymer chitosan increases liposome stability and prevents leaking. This biopolymer also boosts medication efficacy by ensuring continuous release.[5] Cholesterol is a structural component of membranes and a precursor to several steroids. Lipids include cholesterol. It is a component of cell membranes that reduces permeability and increases density.[6] Polyethylene glycol is a popular synthetic polymer due to its high biocompatibility and hydrophilicity. It is widely used to stabilize polymers due to its biocompatibility, solubility in aqueous and organic mediums, non-toxicity, low antigenicity and immunogenicity, and good excretion and kinetics.[7]

The objective of our study is to design and characterize pitavastatin-loaded liposomes by ether injection method using 3^2 factorial design. In our work, several polymers including chitosan, lecithin, cholesterol, and polyethylene glycol-6000 were used to make pitavastatin-loaded liposomes. These polymers would enhance bioavailability by increasing solubility over a prolonged period.

MATERIALS AND METHODS

Materials. Pitavastatin calcium (Beximco Pharmaceuticals Limited., Bangladesh), Lecithin (Beximco Pharmaceuticals Limited, Bangladesh), Cholesterol (Alfa Aesar, Great Britain, UK), Diethyl ether (Merck, Germany), Methanol (Merck, Germany), Chitosan (Merck, Germany), PEG 6000 (Merck, Germany).

Preparation of liposome. Cholesterol and lecithin were dissolved in 8 mL of diethyl alcohol, which was then mixed with 2 mL of methanol containing a calculated amount of pitavastatin calcium. The resulting solution was progressively injected into 20 milliliters of phosphate buffer (pH 7.4) using microneedles at 1ml/min. A magnetic stirrer was used to constantly mix the solution. The temperature was kept between 60°C and 65°C. Temperature differences between the phases caused fast vaporization of the ether, which resulted in spontaneous vesiculation and the creation of liposomes while the liquid lipid solution was in the aqueous phase. All formulations were created in line with the experimental design by utilizing the same technique and varying the amounts of lecithin, cholesterol, chitosan, and PEG-6000, as shown in table 1 and table 2.

Table 1. Independent variables and their levels in experimental design.

Independent variables	Levels (Actual code)	
	Low (-1)	High (+1)
X1: Lecithin	100	150
X2: Cholesterol	75	100
X3: Chitosan/ PEG-6000	15	25
Dependent variables	**Goals**	
Y1: Drug entrapment efficiency (%)	Maximize	
Y2: CDR at 8 hrs. (%)	Minimize	

Determination of drug entrapment efficiency. The amount of drug that was encapsulated in liposomes was assessed by centrifuging unentrapped drugs from the dispersion of the liposomes and then analyzing the vesicle for the amount of drug and consequently, entrapment effectiveness.[8] For 2 hours, the suspension of liposomes (2 milliliters) was spun

with 4000 rpm. The supernatant was collected after centrifugation. The recovered supernatant was spectrophotometrically examined at 245 nm to calculate drug entrapment efficiency. The following formula was used to calculate the amount of drug entrapped in liposomes.[9]

$$(\%)\ \text{Drug Entrapment Efficiency} = \frac{\text{Weight of entrapped drug}}{\text{Weight of total amount drug}} \times 100$$

Table 2. Formulations of different polymers using factorial design.

Formulation Code	Drug	Lecithin	Cholesterol	Chitosan	PEG 6000	DEE	CDR
	(mg) actual code with level					%	
FLE-1	5	100 (-1)	75 (-1)	-	-	88.78	68.12
FLE-2	5	150(1)	100 (1)	-	-	80.29	71.39
FLE-3	5	100 (-1)	100 (1)	-	-	83.37	80.19
FLE-4	5	150(1)	75 (-1)	-	-	90.23	70.29
FCH-1	5	-	75 (-1)	15 (-1)	-	92.73	79.20
FCH-2	5	-	100 (1)	25 (1)	-	85.49	88.70
FCH-3	5	-	100 (1)	15 (-1)	-	88.68	86.32
FCH-4	5	-	75 (-1)	25 (1)	-	94.44	78.01
FPEG-1	5	-	75 (-1)	-	15 (-1)	88.98	68.12
FPEG-2	5	-	100 (1)	-	25 (1)	83.90	70.53
FPEG-3	5	-	100 (1)	-	15 (-1)	85.42	78.91
FPEG-4	5	-	75 (-1)	-	25 (1)	90.51	66.22

F=Formulation, LE=Lecithin, CH=Chitosan, PEG=Polyethylene glycol.

***In vitro* release kinetic model**. *In vitro* drug release data were included in a number of kinetic models. Zero-order, first-order, Higuchi, and Korsmeyer-Peppas kinetic models were used.[10-12] To ascertain the mechanism of drug release, we evaluated the coefficients of correlation (R^2) values of the linear curves from several regression models.

Successive fractional dissolution time. To characterize the rate of release under various experimental settings, MDT (Mean Dissolution Time) and T25, T50 and T80% were determined. MDT measurement was used to determine the dosage form's rate of drug release and the polymer's effectiveness.[13]

Permeability studies

***In vitro* permeability study using cellulose dialysis membrane tubing.** At room temperature, the cellulose membrane was cut into pieces that were 9 cm long. Then, it was put in a large amount of distilled water (1L) for 30 minutes. To clean the membrane, it was rinsed with distilled water very well. The pure drug solution and different liposomes (1.5 ml) were taken and injected into the end of the dialysis tubing that was sealed with the disposable 5 mL plastic syringe. Then, the other end of the tubing was closed by sewing with thread. The dialyze-filled tubing was placed in about 900 mL of dialysis media of phosphate buffer (pH 7.4). The tube of the sample was allowed to spin inside the dissolution vessel of the USP II (paddle) dissolution test apparatus, which was filled with the dialyzing medium and kept at 37 ± 0.5°C and stirred at 75 rpm. Then, 10 mL samples were withdrawn at predetermined time intervals with the replacement of a fresh dialyzing medium. Withdrawn samples were analyzed for drug content at 245 nm for pitavastatin by UV spectrophotometer.[10]

***Ex vivo* permeability investigation using chicken intestinal sac**. Chicken intestinal sac was

collected and rinsed with Krebs- Ringer solution. Then 6 cm long cutting sacs were prepared by tying up one end of the intestinal segments with thread. *Ex vivo* diffusion study was conducted using chicken intestinal sac in the same manner used in vitro studies. Finally, the permeability of pure drug and different formulations was analyzed by determining the permeability coefficient using the Fick's law of diffusion

$$\frac{dQ}{dT} = DAh\,(Co\text{-}Cp)$$

Here, $\frac{dQ}{dT}$=Rate of drug diffusion, D=Diffusion co-efficient, A=Area of intestinal sac, H= Thickness of the intestinal sac. The equation can be simply written as-

$$\frac{dQ}{dT} = D$$

Stability analysis. To determine the stability of liposomal formulations, samples were held at 2–8°C (refrigeration), 25°C/60% RH and 40°C/75% RH (relative humidity), for 90 days at the laboratory of the University of Asia Pacific. The drug content of all formulations was determined at monthly intervals using the approach described earlier in the entrapment efficiency parameter.[14-15]

Determination of particle size. Vesicle size determination is a crucial characteristic to consider when examining how formulations are impacted by excipients. The Zetasizer was used to measure the particle size of liposomes dispersed systems from sub-nanometer to several micrometers in diameter, using the technique of Dynamic Light Scattering (DLS). [16]

Zeta potential analysis. In a beaker, the liposome suspension was diluted with distilled water while being constantly stirred with a magnetic stirrer. The Zetasizer was used to determine the zeta potential (3000 HAS, Malvern Ltd, UK). According to DVLO theory, the stability of suspended particles is determined by the total potential energy function VT, which is defined as VT=VA+VR+VS.

where VA denotes the attractive energy resulting from van der Waals interactions, VR denotes the repulsive force resulting from the electrical double layer of particles, and VS denotes the solvent's potential energy.[17]

Fourier transform infrared spectroscopy (FIIR). The drug, cholesterol, and lecithin interactions were conducted utilizing an FT-IR Spectrophotometer. The spectrum was acquired between the wavelengths 4000-400 cm^{-1}. Individual samples of pitavastatin, cholesterol, chitosan, and PEG-6000 was dispersed by compressing them into discs using a hydraulic press for 5 minutes. The pellets were put in the path of the light and the spectra were captured. Using dry potassium bromide pellets, a baseline correction was performed.

Scanning electron microscope (SEM) examination of the surface morphology. The surface structure of liposomes was investigated using a scanning electron microscope (JEOL, JSM-6490 LA, Japan).[14] Before the inspection, the liposome suspension was thoroughly dried and SEM was performed at various magnifications of 20.0 kv 75, 20.0 kv 90, 20.0 kv 95, 20.0 kv 140, 20.0 kv 300, 20.0 kv 600, and 20.0 kv 1000.

Factorial design statistical analysis. ANOVA was used to analyze the data. Eight experimental runs (Design Expert® software-Trial Version 7.1.6, Stat-Ease Inc., MN) were constructed by varying two parameters (lecithin and cholesterol content) (low and high). The model's regression parameters and graphical interpretation for statistically significant answers ($p \leq 0.05$) were calculated by a design expert.

Optimization by desirability function. Optimization is used to convert the multi-response characteristics into single–response characteristics. In this study, several formulations were optimized at the same time using the optimization method in the design of experience (DOE) software and a desirability function. Several articles have talked about the desirability function approach to optimizing multiple responses in the past few months. Any answer that isn't within the range asked for is completely unacceptable.[14,18]

RESULTS AND DISCUSSION

Formulation development of pitavastatin-loaded liposomes by 3^2 factorial design. The pitavastatin-loaded liposomes were prepared using 3^2 factorial designs mentioned in the method section. All formulations were prepared to study the effect of three independent variables such as X1 (amount of lecithin), X2 (amount of cholesterol), and X3 (amount of chitosan/ PEG-6000) on two dependent variables Y1 (% DEE), and Y2 (% CDR), respectively as shown in table 1. Prepared liposomes were characterized by % DEE, % CDR, FTIR, SEM and zeta potential, etc. *In vitro* and *ex vivo* permeability studies were also done. Statistical analysis was performed using ANOVA.

Drug entrapment efficiency. The drug entrapment efficiency has been depicted in figure 1**.** According to figure 1, the minimum and maximum percent of drug entrapment efficiency (% DEE) of pitavastatin-loaded liposomes were 80.29 and 90.23 percent; 85.49 and 94.44 percent, and 83.9 and 90.51 percent for FLE-2 and FLE-4; FCH-2 and FCH-4 and FPEG-2 and FPEG-4, respectively.

Table 2 and figure 1 show that the maximum concentrations of lecithin and cholesterol increased the efficiency of drug entrapment. Cholesterol decreases the leakage of drugs from the liposomes by enhancing their fluidity. However, raising the content of cholesterol over the threshold can disrupt the membranes of the liposomes' regular linear structure, which can reduce the effectiveness of entrapment.[6] It was also observed that the addition of chitosan and PEG-6000 affected the effectiveness of drug capture, especially in two results. Furthermore, the effectiveness of drug trapping was increased by increasing the amount of chitosan and PEG-6000 in formulations. This is likely due to the fact that chitosan and PEG-6000 were utilized to coat the outer layer of the bilayer made of lipids which increased the amount of drug trapped inside the bilayer.

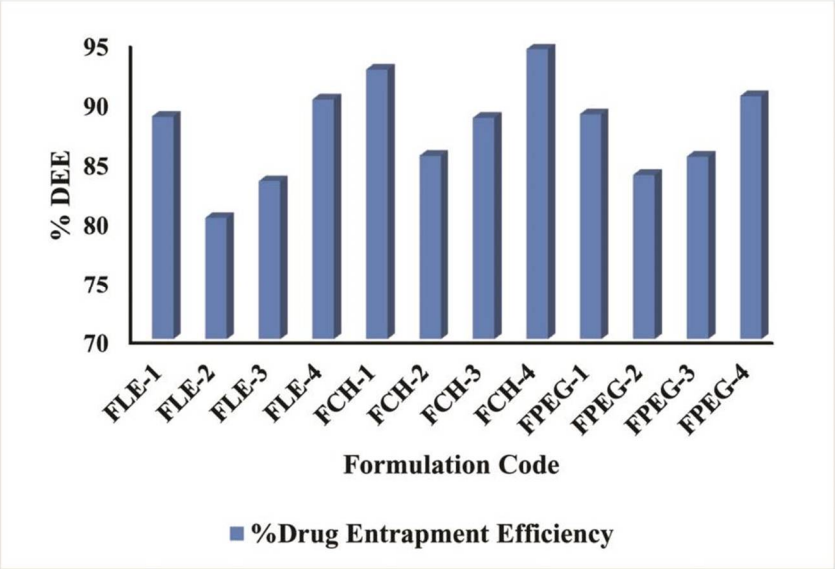

Figure 1. Percent drug entrapment efficiency (% DEE) of liposomes.

***In vitro* drug release studies.** After 8 hours of dissolution, the lowest and highest drug releases were 72.19 and 89.32 percent, 71.12 and 87.29 percent, and 70.12 and 85.29 percent, for FCH-2 and FCH-4, FLE-2 and FLE-4, and FPEG-2 and FPEG-4, respectively. Figure 2 shows that increasing the cholesterol and lecithin concentrations reduces drug release from the formulation, which may be due to

the increased stiffness of the vesicles. Furthermore, chitosan-based formulations demonstrated a larger percentage of drug release than lecithin- and PEG-6000-based formulations, which could be attributed to chitosan's ability to thin liposomal vesicles and increase drug solubility. PEG-6000-based formulations released medicines more slowly and efficiently than other formulations, which could be attributed to the integration of PEG-6000 into vesicles.

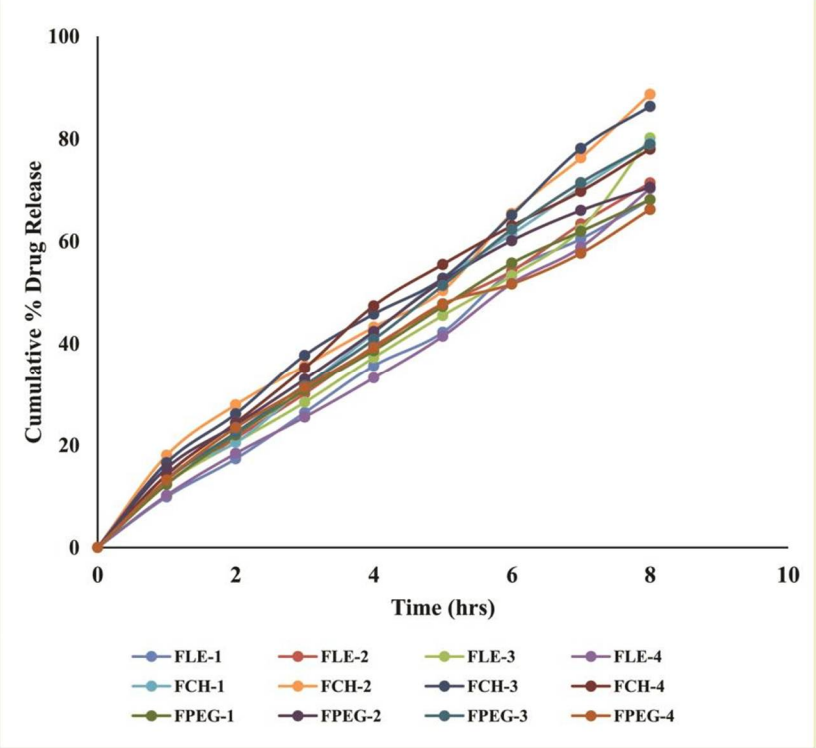

Figure 2. Percent release kinetic plot of different formulations of liposomes.

Table 3. Interpretation of release rate constants (n) and R^2 values for different release kinetics of different liposomal formulations.

Formulation Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	K0	R^2	K1	R^2	KH	R^2	n	R^2
FLE-1	8.562	0.998	-0.140	0.978	25.061	0.920	0.214	0.927
FLE-2	8.755	0.996	-0.152	0.976	25.950	0.942	0.325	0.945
FLE-3	8.583	0.997	-0.145	0.975	25.338	0.936	0.245	0.936
FLE-4	8.229	0.998	-0.131	0.978	24.107	0.922	0.348	0.928
FCH-1	9.819	0.997	-0.189	0.966	28.907	0.930	0.256	0.979
FCH-2	10.326	0.987	-0.237	0.882	30.432	0.924	0.246	0.958
FCH-3	10.384	0.993	-0.207	0.927	30.756	0.938	0.320	0.983
FCH-4	9.605	0.988	-0.161	0.987	28.820	0.957	0.344	0.958
FPEG-1	9.069	0.998	-0.164	0.965	26.761	0.935	0.245	0.958
FPEG-2	10.271	0.997	-0.216	0.932	30.167	0.927	0.320	0.969
FPEG-3	10.384	0.998	-0.212	0.929	30.118	0.916	0.252	0.945
FPEG-4	9.402	0.997	-0.175	0.963	27.742	0.934	0.363	0.927

The data in figure 2 demonstrated the pattern of release of pitavastatin-loaded liposomes. The statistical level of significance ($\alpha = 0.05$) was applied to the release date, and they were found to be statistically significant in all cases where the "p" value was less than 0.05, as shown in table 6a.

Release rate constants (n) and correlation coefficient (R^2) values study. The release rate constants (n) and correlation coefficients (R^2) for fifteen formulations of pitavastatin-loaded liposomes with various release rate kinetics are shown in table 3. As the release exponent (n) was smaller than 0.43, the most likely mode of drug release was fickian (class I) diffusion.

Successive fractional dissolution time. Dissolution data were utilized to characterize the drug release rates under various experimental circumstances. Figure 3 shows the results of determining the formulations' T25, T50, T80, and MDT values. Drug release from liposomes is characterized by T25, T50, T80, and MDT values. A higher MDT value indicates a polymer's ability to retain medicines and *vice versa*. The figure depicts the effect of excipient concentration on T25, T50, MDT, and T80% values, as well as their characteristics. The MDT value is also dependent on polymer loading, polymer type, and drug molecule physicochemical characteristics.

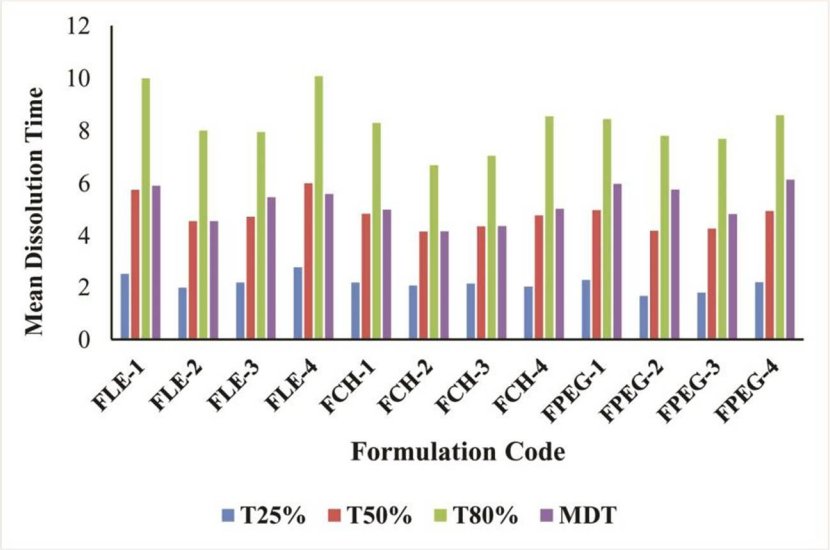

Figure 3. Successive fractional dissolution time (hour) of pitavastatin-loaded liposomes.

***In vitro* and *ex vivo* permeability studies.** Permeability studies were conducted *in vitro* and *ex vivo* to determine the effect of pure drug and formulated liposomes on permeability augmentation or diffusion. As illustrated in figures 4a and 4b, pitavastatin-loaded liposomes exhibited greater diffusion than pure medication after 8 hours. The use of chitosan as a surfactant enhanced the drug's absorption across epithelial layers. According to reports, chitosan's cationic nature enables it to open tight connections in a cell membrane. Drug permeability followed the same sequences in all cases: FCH-4> FLE-4> FPEG-4> pure drug (PD).

Stability studies. To determine the stability of various liposomal formulations such as FCH-4, FLE-4, and FPEG-4, they were stored at three different temperatures for 90 days in accordance with the ICH recommendations listed in table 4. For stability testing, the physical parameters were fixed for formulations that demonstrated the maximum drug entrapment efficiency. The produced liposome is stable and exhibits little change in drug entrapment efficiency.

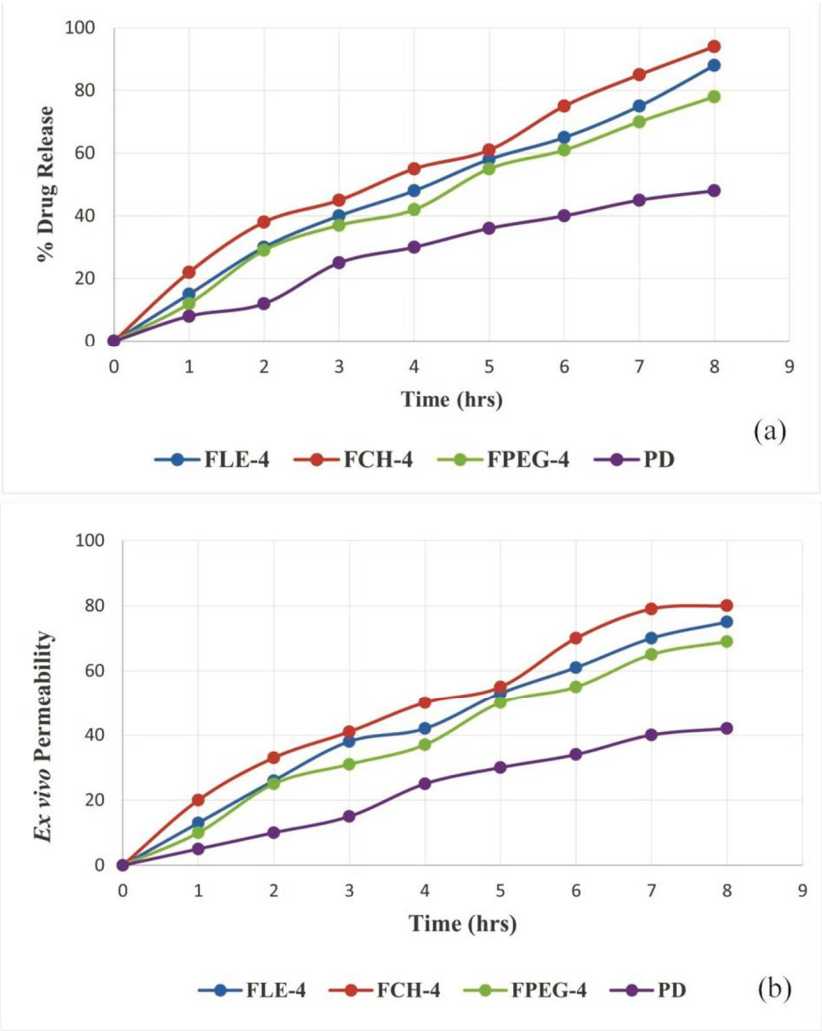

Figure 4. Permeability study of pitavastatin loaded liposomes a) *in vitro* release plot and b) *ex vivo* release plot respectively.

Table 4. Stability studies by observing DEE % of liposomes.

Storage conditions	Initial (mean ± SD, % RH)			30 days (mean ± SD, % RH)			90 days (mean ± SD, % RH)		
	FCH-4	FLE-4	FPEG-4	FCH-4	FLE-4	FPEG-4	FCH-4	FLE-4	FPEG-4
2℃–8℃ (Refrigeration)	94.4±0.4	90.2±0.3	88.4±0.2	94.1±0.1	90.1±0.5	88.1±0.3	93.1±0.2	89.2±0.9	87.1±0.2
25℃/60% RH	94.3±0.5	89.2±0.1	86.2±0.1	94.2±0.3	88.7±0.8	85.2±0.1	93.9±0.8	87.1±0.5	84.2±0.1
40℃/75% RH	94.1±0.1	89.8±0.4	85.3±0.6	93.3±0.4	88.1±0.1	84.1±0.8	91.1±0.1	86.1±0.2	83.1±0.9

Particle size analysis. The particle size distribution of liposomes from various formulations at various cholesterol, lecithin, chitosan, and PEG-6000 concentrations is shown in table 5.

Table 5 shows that the liposomal formulation's mean particle size ranges from 3.51 µm to 4.19 µm. Cholesterol was found to directly increase the particle size of liposomes. Cholesterol and phospholipids

should pack closely together at low cholesterol levels, increasing curvature and lowering flexibility. The rigidity of the bilayer membrane rises with cholesterol levels, preventing shrinkage and resulting in bigger vesicles. Liposomes made of PEG-6000 were larger than those made of chitosan and lecithin.

Table 5. Effect of excipients on the particle size of liposomes.

Formulation code	Mean particle size (µm)
FCH-2	3.51±0.03
FCH-4	3.74±0.11
FLE-2	3.93±0.17
FLE-4	3.84±0.14
FPEG-2	4.19±0.06
FPEG-4	4.13±0.11

Zeta potential analysis. According to figure 5, the zeta potentials of all formulations ranged between -29.2 and -35 mV. The results established that there is no possibility of liposomal vesicle coalescence and that manufactured liposomes have acceptable physical stability. The graphic demonstrated that the independent factors that reduced the size of droplets also raised their charge. Indicator of the liposome suspension's surface charge and stability. Zeta potentials above +30 mV and below -30 mV indicate significant resistance to coalescence.

Fourier transforms infrared spectroscopy (FTIR). According to figure 6, the primary functional group peaks are within the range of previously published values for both the pure drug and the formulation, showing that there are no drug-polymer interactions. There were no identifiable peaks in the FTIR spectra of pitavastatin calcium-loaded liposomal formulations like FCH-4. This demonstrated that no interaction occurred between the medication and the excipients used to produce the liposomes.

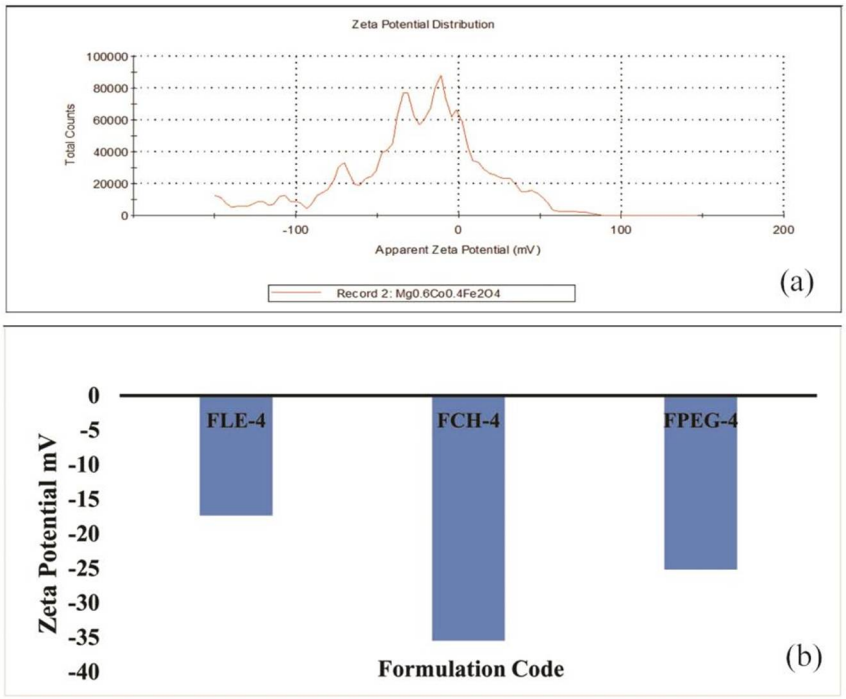

Figure 5. Zeta potential of pitavastatin-loaded liposomal formulations a) FCH-4 and b) graphical presentation, respectively.

Scanning electron microscopy (SEM) of the pitavastatin-loaded liposome. SEM was used to examine the liposomes' surface morphology. Figure 7 depicts the picture of FLE-4. The surface morphology of liposomal particles reveals that they are distinct and spherical in shape with an uneven

surface due to the encapsulated drug. The micrograph demonstrates that liposomes with a diameter of 50–100 m were created.

Statistical analysis using ANOVA. As shown in tables 6a and 6b and figure 8, an expert-designed factorial design was used to establish statistical significance. For each response (Y1 and Y2), the ANOVA test yielded p values less than 0. 0001. This model fits all replies (Y1 and Y2) ($p \leq 0.05$). The 'lack of fit' test found that all responses fitted into the quadratic model had a non-significant lack of fit ($p>0.1$). FCH-4 had an R^2 of 94.6 percent, FLE-4 had an R^2 of 91.9 percent, and FPEG-4 had an R^2 of 90.9 percent.

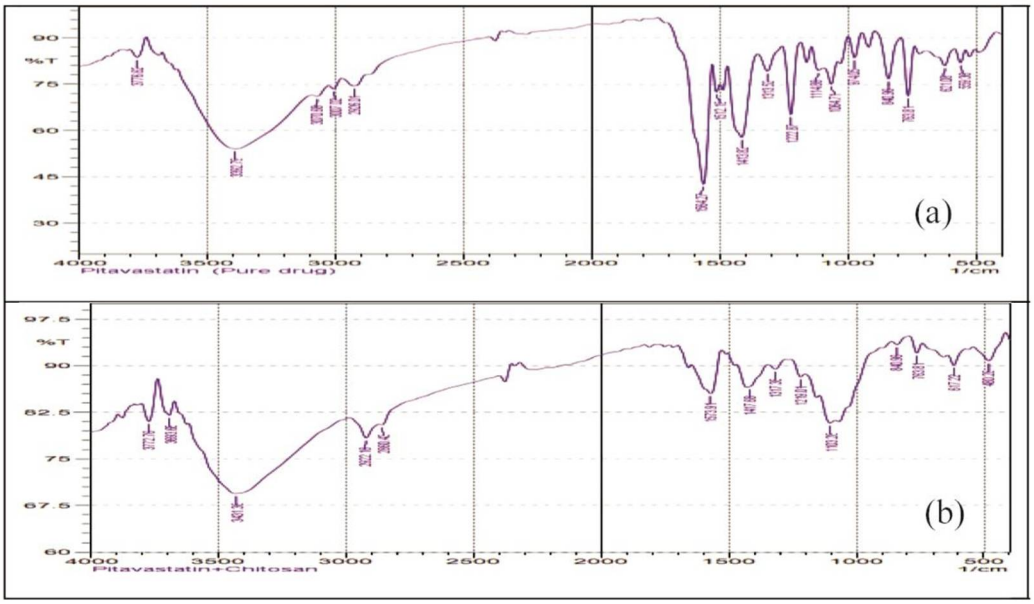

Figure 6. FTIR image on a) Pure drug and b) FCH-4

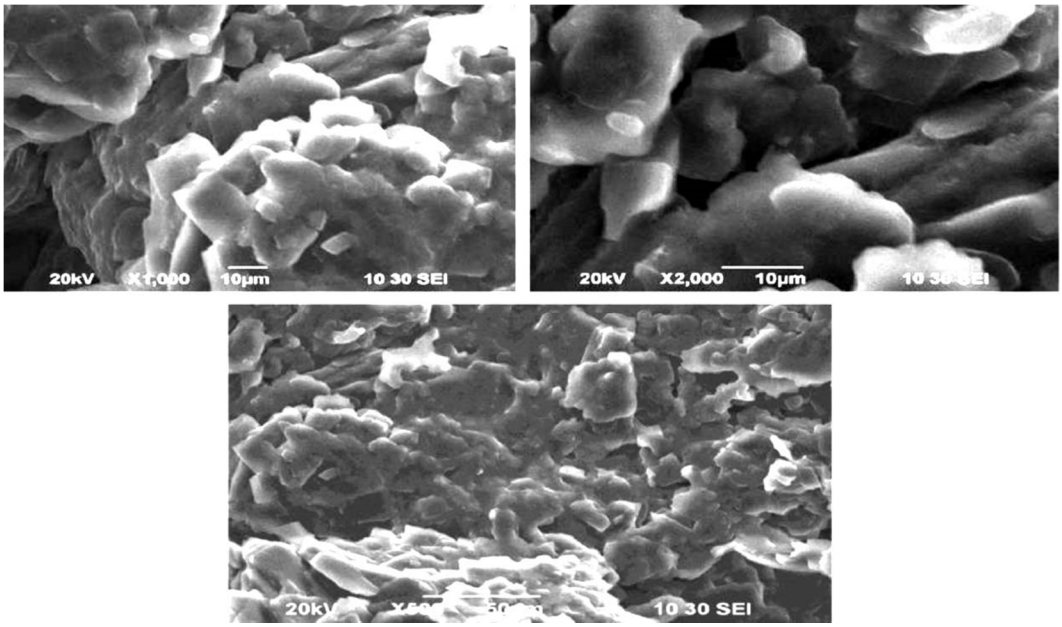

Figure 7. SEM studies of pitavastatin liposomal formulations FLE-4.

Table 6a. ANOVA and lack of fit tests of the quadratic model.

Run	Response		F Value	Probability > F	Comment
FCH-4	Y1	Model	75.33	< 0.0001[a]	Significant
		Lack of Fit	4.14	0.2095[b]	Not significant
	Y2	Model	266.19	< 0.0001[a]	Significant
		Lack of Fit	0.42	0.8539[b]	Not significant
FLE-4	Y1	Model	76.32	< 0.0001[a]	Significant
		Lack of Fit	1.12	0.4940[b]	Not significant
	Y2	Model	91.75	< 0.0001[a]	Significant
		Lack of Fit	0.29	0.9437[b]	Not significant
FPEG-4	Y1	Model	97.07	< 0.0001[a]	Significant
		Lack of Fit	0.35	0.9129[b]	Not significant
	Y2	Model	98.65	< 0.0001[a]	Significant
		Lack of Fit	0.17	0.9875[b]	Not significant

[a]Significance probability values (Probability>F) less than 0.05 implies that the model is significant; [b] Non-significant lack of fit (p-value > 0.1) proves the adequacy of model fit.

Table 6b. Regression analysis of the responses (Y1 and Y2) of different formulations.

Run	Quadratic model	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	SD	CV (%)
FCH-4	Y1	0.946	0.971	0.880	18.06	0.92	1.07
	Y2	0.888	0.969	0.952	25.53	2.60	0.56
FLE-4	Y1	0.919	0.829	0.757	17.97	1.09	1.21
	Y2	0.861	0.881	0.824	15.51	4.44	1.27
FPEG-4	Y1	0.909	0.941	0.917	29.86	1.00	1.13
	Y2	0.842	0.998	0.993	101.15	0.75	0.22

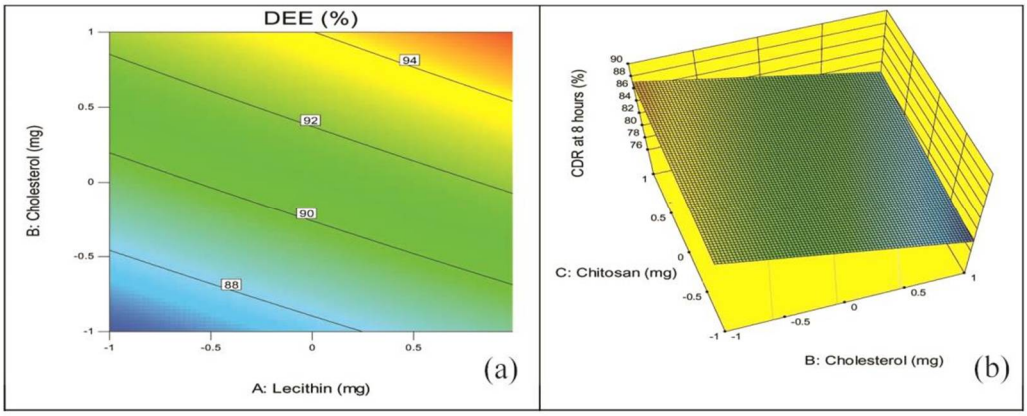

Figure 8. a) Contour plots showing the impacts of X1, X2 and X3 on percent drug entrapment efficiency and b) surface response 3D curve showing the impacts of X1, X2, and X3 on the percent of cumulative drug release.

Response surface and contour plot analysis. On the other hand, Figure 8 shows three-dimensional response surface plots and two-dimensional contour plots of responses across selected components. These graphs show how one variable's level impacts the effect of another. Because these graphs can only express two variables, one must always be fixed.

Optimization. The independent variables were simultaneously optimized for all three formulations using the desirability function after evaluating the impact of the dependent (X1, X2, and X3) and

independent (Y1 and Y2) variables on the formulations. Y1 and Y2 were set to be maximized among the formulas. Table 7 shows the desirability value derived by the Design-Expert software.

Table 7. Calculated values for optimized different liposomes.

RUN	X1	X2	X3	Y1	Y2	Desirability
FCH-4	150	100	25	94.44%	88.01%	0.992
FLE-4	145	95	25	91.10%	86.09%	0.924
FPEG-4	135	90	25	90.01%	83.02%	0.914

CONCLUSION

Liposomal drug delivery appears to be an amazing method to overcome the problems that arise from the oral administration of pitavastatin. Liposomes containing pitavastatin showed superior performance over pure drugs in relation to dissolution profiles, *in vitro* and *ex vivo* permeability studies. Zeta-potential ranged between -29.2 and -35 mV demonstrating the high stability of the liposomal suspension. The analysis parameters may be changed substantially by altering the excipients and polymer. So, the pitavastatin-loaded liposomal drug delivery systems could be a possible monitored drug delivery device to aid in treating hyperlipidemia by providing increased solubility and bioavailability.

ACKNOWLEDGMENT

The authors would like to thank Beximco Pharmaceuticals Limited., Bangladesh for supplying active components and the University of Asia Pacific for providing research space.

CONFLICT OF INTEREST

The authors claim that they do not have any competing interests.

REFERENCES

1. Cho, K., Wang, X. and Nie, S. 2008. Therapeutic nanoparticles for drug delivery in cancer. *Clin. Cancer Res.* **14**, 1310-1316.
2. Tiwari, G., Tiwari, R., Bannerjee, S., Bhati, L., Pandey, S. and Pandey, P. 2012. Drug delivery systems: an updated review. *Int. J. Pharma Invest.* **2**, 2-11.
3. Moghadasian, M.H. 1999. Clinical pharmacology of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Life Sci.* **65**, 1329-1337.
4. Greenwood, J.S. and Zamvil, S.S. 2006. Statin therapy and autoimmune disease: from protein prenylation to immunomodulation. *J. Nat. Rev. Imm.* **6**, 358-370.
5. Bozzuto, G. and Molinari, A. 2015. Liposomes as nanomedical devices. *Int. J. Nano Med.* **10**: 975-999.
6. Liu, J.P. 2009. Cholesterol: a review. *Mol. Cell Endo.* **303**, 1-6.
7. Yamaoka, T., Tabata, Y. and Ikada, Y. 1994. Distribution and tissue uptake of poly (ethylene glycol) with different molecular weights after intravenous administration in mice. *J. Pharm Sci.* **83**, 60-606.
8. Laouini, A., Jaafar, M., Blouza, C., Sfar, S., Charcosset, C. and Fessi, H. 2012. Preparation, characterization, and applications of liposomes: state of the art. *J. Coll. Sci. Biotech.* **1**, 147-168.
9. Taha, E.I., Anazi, M.H., Bagory, I.M. and Bayomi, M.A. 2014. Design of liposomal colloidal systems for ocular delivery of ciprofloxacin. *Saudi Pharma J.* **22**, 231-239.
10. Dewan, I., Islam, S. and Rana, M.S. 2015. Characterization and compatibility studies of different rate retardant polymer loaded microspheres by solvent evaporation technique: *In vitro-in vivo* study of vildagliptin as a model drug. *J. Drug Deliv.* 1-12.
11. Higuchi, T. 1963. Mechanism of sustained-action medication: theoretical analysis of rate of release of solid drug dispersed in solid matrices. *J. Pharm Sci.* **52**, 1145-1149.
12. Ritger, P.L. and Peppas, N.A. 1987. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. *J. Con. Rel.* **5**, 37-42.
13. Mockel, J.E. and Lippold, B.C. 1993. Zero-order drug release from hydrocolloid matrices. *Pharm Res.* **10**, 1066-1070.
14. Dewan, I., Nahar, S., Islam, S.M.A., Khandaker, S.I., Nasreen, W. and Hoque, O. 2018. Formulation and evaluation of metoprolol tartrate loaded niosomes using 2^3 factorial design. *J. Pharma Res. Int.* **22**, 1-17.
15. Nisu, S.B.N., Karmoker, J.R., Ali, F.F., Rafa, N.N., Hoque, O. and Dewan, I. 2018. *In vitro* and *ex vivo* studies of linagliptin loaded non-ionic surfactant vesicles using statistical optimization. *J. Adv. Med. Pharm Sci.* **18**, 1-16.
16. Ho, R.J.Y., Rouse, B.T. and Huang, L. 1986. Target-sensitive immunoliposomes-preparation and characterization. *J. Bio.* **25**, 500-506.
17. Kadu, P.J., Kushare, S.S., Thacker, D.D. and Gattani, S.G. 2011. Enhancement of oral bioavailability of atorvastatin calcium by self-emulsifying drug delivery systems (SEDDS). *Pharm Dev. Tech.* **16**, 65-74.
18. Singh, B., Kumar, R. and Ahuja, N. 2005. Optimizing the drug delivery systems using systematic design of experiments. Part I: Fundamental aspects. *Cri. Rev. Therapy Drug Carr. Sys.* **22**, 27-105.