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Formulation, *ex-vivo* and *in-vitro* characterization of liposomal drug delivery system of Fexofenadine

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ABSTRACT

In order to improve the low permeability associated with conventional or immediate release dosage forms such as nasal spray and oral solid dosage form, tablets, fexofenadine liposomes were prepared by using ethanol injection method by varying the concentration of surfactant, (Tween -80), along with crude lecithin and cholesterol. The optimized formulations was evaluated for size, shape, entrapment efficiency and *in vitro* drug release. In this study the optimized formulated liposomes appeared to be spherical in shape and particle size was found to be 4703.667±445 nm. There is 1.25 times more flux of liposome as compared with free drug. The entrapment efficiency was found to be 65% and *in vitro* permeation was found to be 4.535% in 5 hrs. Fexofenadine liposome (optimized formulation) was formulated with Tween 80, Crude lecithin and cholesterol in the amount of 5ml, 8gm and of 2.5gm respectively which was found to be promising in drug permeation study. The zeta potential was found to be between -15.86 to -28.14 mV which proved liposome particles are well ispersed and stable. Liposome entrapped with fexofenadine was formulated, optimized and various parameters were evaluated successfully.

Keywords: Liposomes, Lecithin, Fexofenadine

INTRODUCTION

Biopharmaceutical classification system (BCS) class III drugs are having high solubility but low permeability. Bioavailability can be enhanced by formulating dosage form of this class of drugs on lipid based. Formulation excipients do not play a great role to overcome low permeability of this class.[1] Thus, permeation as a rate limiting factor for bioavailability can only be overcame by formulating such drugs in lipid-based dosage form like liposome rather than adding formulation excipients. Lecithin-based liposomal delivery increases plasma drug concentration which will increase bioavailable dose in body as a consequence less drug dose can attain therapeutic window and show therapeutic effect.[2]

Liposomes were discovered by Alec D Bangham in the 1960s at the Babraham Institute, University of Cambridge, and consisted of single or multiple concentric lipid bilayers encapsulating an aqueous compartment.[3] It can be formulated using both natural and synthetic lipids and surfactants. Liposomes consisted of lipid belayed like cellular membranes and because of their ability to incorporate various substances; it is considered an ideal drug carrier because it has ability to permeate through membranes.

Fexofenadine is an antihistamine used in allergy symptoms such as watery eyes, runny nose. It works by blocking histamine that your body makes during an allergic reaction. It is a poorly absorbable drug and has been formulated in intranasal dosage forms[4-7] to reduce the dose by increasing bioavailability of fexofenadine. The maximum adult dose of fexofenadine is 120 mg–180 mg/once a day through post oral (P.O.). Though this much dose is recommended bioavailable dose of this drug is very less than this because this drug falls under BCS class III (high solubility; poor permeability). Thus, increasing permeation can reduce, the dose required to show therapeutic effect. The rate limiting factor for bioavailability of this drug is permeation. We can enhance dissolution by adding excipients in formulation or using different techniques, but these will not help to enhance permeation or drug absorption.

This study aims to enhance permeation of BCS class III drug (fexofenadine) using lipid-based dosage form (liposome delivery) through P.O. to assess the possibility to formulate this drug in liposomal drug delivery.

MATERIALS AND METHODS

Materials

All the chemicals used were of analytical grade [Table 1]. Lecithin was extracted because it was not available in local market which has to be imported and takes time. This study has to be finished within 6 months.

Methods

Extraction of lecithin from egg yolk[8]

The egg yolk lecithin is a functional lipid found in yolk of egg which contains phosphorous in structure. Because of this, it is also known as Phospholipids and is used as a natural emulsifier food, cosmeceuticals and pharmaceuticals and is safe.

The extraction of lecithin from egg yolk [Figure 1].

Experimental design

Three independent factors (lecithin, Tween 80 and Cholesterol) two levels (3^2) Central composite design (CCD) was created using Minitab 18 software for optimization of three independent variables [Table 2]. Fixed amount of drug (100 mg) was used in all formulation. Dependent variables were taken as a Drug loading capacity. The quantity of the three independent factors was optimized using contour plots drawn from the data obtained from experiments.

Preparation of liposomes

Ethanol injection is one of the techniques frequently used to produce liposomes which favor both simplicity and safety.[9] Ethanol injection method was employed for the preparation of liposome.[9-15] The method involves the dissolution of lecithin

Table 1: List of materials used in this study and their manufacturers

S. No.	Name of chemical	Manufactures
1	Fexofenadine	Gift sample
2	Ethanol	S.D. fine chem. ltd, Bojsar
3	Acetone	Qualigens fine chemical, India
4	Tween 80	Merck fine chemical
5	Petroleum ether	Merck fine chemical
6	Egg	Local grocery
7	Crude lecithin	Naturally extracted
8	Chloroform	Qualigens fine chemical, India
9	HCL	Merck fine chemical
10	Goat intestine	Local cold store

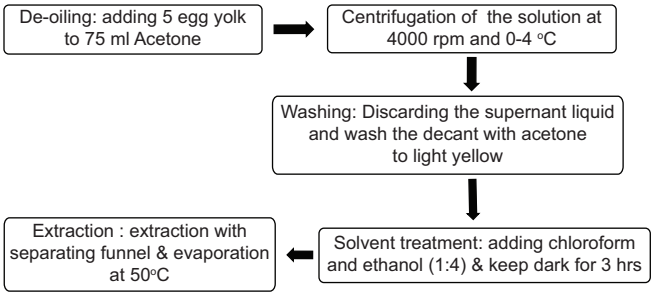

Figure 1: Flow diagram of extraction of Lecithin from Egg Yolk

Table 2: 3^2 CCD design of experiment and Drug loading in percentage

Formulation	Lecithin (mg)	Tween 80 (mg)	Cholesterol (mg)	Drug Loading (%)
F1	4.5	1.5	2.5	33.29
F2	4.5	1.5	2.5	33.18
F3	9	3	0	23.82
F4	0	0	5	6.60
F5	0	3	5	0
F6	4.5	1.5	2.5	0
F7	4.5	1.5	0	23.23
F8	9	0	5	52.52
F9	4.5	0	2.5	29.32
F10	0	0	0	7.85
F11	0	3	0	10.89
F12	9	0	0	7.41
F13	4.5	4	2.5	37.60
F14	0	1.5	2.5	0.39
F15	4.5	1.5	2.5	0
F16	4.5	1.5	2.5	0
F17	12	1.5	2.5	0
F18	4.5	1.5	6.7	37.11
F19	9	3	5	0
F20	4.5	1.5	2.5	0

into an ethanol (organic phase) and further dispersion of the ethanolic solution into aqueous solution containing the drug. This immediate dilution of ethanol in the aqueous phase causes the lipid molecules to precipitate and form bi-layer planar fragments which further transform into liposomal system. The various step involved in this experiment for the formation of liposome are highlighted as follow:

Preparation of organic phase

Organic phase was prepared taking respective amount of crude lecithin was tween-80 (surfactant) and cholesterol as per Table 2 and optimized formulation and the volume was maintained up to 15 ml with 99.9% ethanol to dissolve phospholipids.

Preparation of aqueous phase

Fexofenadine (100 mg) was dissolved in 85 ml of 0.01N HCL. Then, the solution was sonicated for 10 min for dissolving the drug completely.

Method of formulation

First of the two phases was prepared Organic phase (Ethanol [15 ml], 99.9%purity) and aqueous phase (85 ml of 0.1N HCl). Composition of the two phases (Organic and aqueous) were prepared as aforementioned. Then the temperature of organic phase was maintained up to 40 ± 2°C for dissolving high amount of crude lecithin in Tween 80. Then, the aqueous phase was maintained up to 60 ± 5°C in water bath. Aqueous phase was kept in water bath to setting temperature 65°C and when 60°C is attained by aqueous phase then the ethanol solution was injected into 85 ml of preheated 0.01N HCL solution (aqueous phase). Then, the resultant suspension was sonicated for 20 min. Liposome formed spontaneously with continuous Sonication. After Sonication, it was centrifuged at 10,000 rpm for 10 min at room temperature. Then, the solution was taken out and the supernatant was removed slowly to avoid the removal of liposome. After the removal of supernatant, few sample was taken with the help of the dropper and kept on a glass slide and observed under microscope at 10× magnification for confirmation of liposomes formation [Figure 2].

Optimization of formulation

After varying the concentration of three independent variables (crude lecithin, tween 80 and cholesterol), the formulation was optimized based on the drug entrapment efficiency percentage. Contour plot was plotted [Figure 3], from which the concentration of crude lecithin and tween 80 was optimized by holding the value of cholesterol. The optimized formulation was prepared with 8 mg of lecithin, 5 ml of tween 80 and 2.5 mg of cholesterol which had drug loading efficacy of 65%.

Zeta potential measurement

Zeta sizer (Malvern ZS) was used to measure zeta potential and liposomes vesicles size were determined operating the equipment at 25°C, water as a medium, duration run was 60 or 80 (s)and the measurement position is 4.65 mm.

Drug loading (%)

Drug loading percentage was determined using Dialysis membrane (DM) (Himedia, India) with molecular weight cut off 12,000 Dalton. Amount of drug trapped was calculated as Total drug used (100 mg) minus drug came out of DM in receiver compartment with 250 ml (0.1N HCl with magnettic stirrer at 150 RPM. Due to MW cut off value of DM liposome can't come out. Thus, the drug trapped in liposome can't come out which is a trapped drug or loaded drug in liposome. Using this Drug loaded (%) was calculated.

Ex-vivo drug permeation study

Ex-vivo drug permeation was carried out using goat intestine (0.18 mm thickness). Fresh goat intestine was procured from slaughter house locally and was stored in −20°C refrigerator. The intestine was thawed at room temperature before used, and then it was dissected and kept on Franz diffusion cell (Orifice diameter=9 mm) in such a way that mucosa (inside of intestine was toward donor compartment) [Figure 4]. Drug permeation was carried out in 7 stations diffusion cell Apparatus, EDC-07 (Electrolab, India) 5 ml drug solution of fexofenadine was kept in donor compartment (5 ml) and Sample (2 ml) was withdrawn from sampling port each hour for 5 h and receiver compartment medium (0.01N HCl) was replaced after each withdrawn. Each cell was thermo-stated and temperature was maintained at37±2°C.

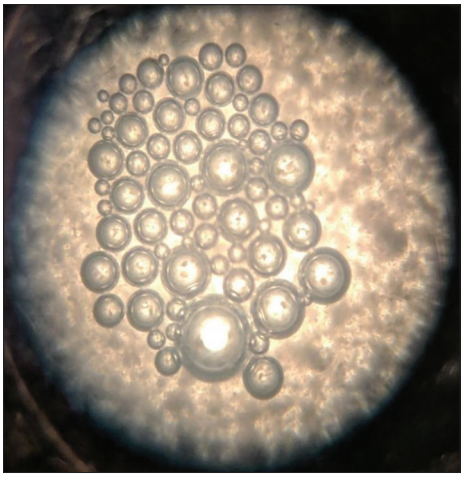

Figure 2: Liposomes vesicles observed under microscope in 10× magnification

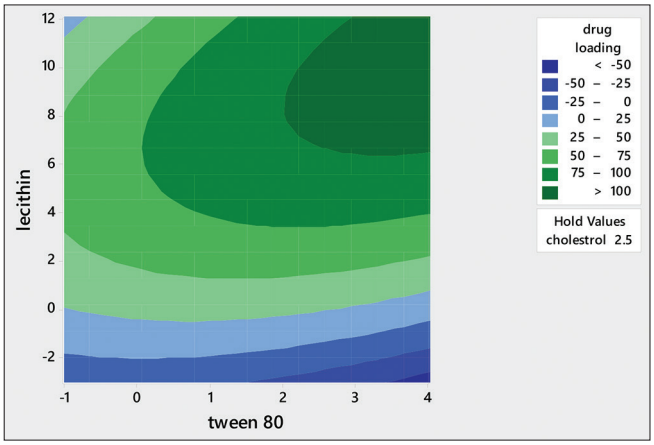

Figure 3: Contour plot of drug loading vs. lecithin, tween 80

Drug quantification

Method validation

UV-visible spectroscopic method was validated as per ICH guidelineQ2 (R1) (reference given below).

Specificity of the method was determined taking reading of placebo and sample (10μg/ml). Different aliquots of drug solutions (5, 10, 15, 20, 25 μg/ml) were prepared and absorbance reading was taken at λ_{max}= 217. The equation and regression coefficient was determined using Excel (Win10) to check the linearity. Accuracy was determined by recovery studies. The experiment was conducted in triplicates of 8, 10, 12 μg/ml, and percentage drug recovery was calculated. For precision, six samples of 10 μg/ml concentration were prepared and percentage RSD was calculated. Robustness of the method was confirmed by small deliberate change in wavelength. Then, single factor analysis of variance (ANOVA) was carried out at 95% confidence interval, and p value was determined.

Linearity

The linear regression was plotted. The value of co-efficient (R^2) and the linear equation was determined for the linearity plot. The plot in a straight line and the linearity result is shown Figure 5.

Accuracy

Accuracy was performed by preparing three different concentrations. The weight of sample was taken so as to make final concentration of the sample after dilution to be about 80% 100% and 120 % respectively. The sample of 0.01 mg/ml was taken as a standard. The percentage recovery is determined by the equation:

Recovery = (analytical result/true value)×100%

RESULT

The spectrum of sample (10 μg/ml) showed peak at 217 but spectrum of placebo showed no peak at 217 nm. This showed the method is specific to the drug. Linearity study showed that the line is straight with (R^2= 0.999) (please give the slope and intercept values also). The percentage drug recovery was all within the specification range (98–102%). Percentage RSD calculation of six determination of 100% of the test concentration (10 μg/ml) of six was found to be <2% (1.02). The % recoveries of drug of 10 μg/ml after analyzing at three wave lenghths (216,217 and 218 nm) were calculated The *P* value was found to be more than 0.05 (0.986299).

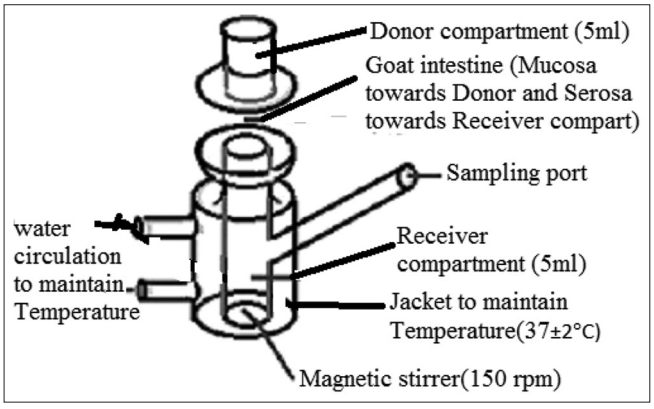

Figure 4: Franz diffusion set up in the experiment

The present work was mainly based on optimizing formulation on the basis of drug loading efficiency by varying the concentration of lecithin, tween 80 and cholesterol. Hence after optimizing formulation, different parameters were evaluated and the following results were obta Drug entrapment efficiency was obtained 65% during optimization. The nature of liposome was found to be spherical overlapped confirmed by the optical microscope (10×) [Figure 2]. The flux of drug in 0.1 HCl and liposome were found to be 0.016 mg/h cm^{-2} and 0.020 mg/h cm^{-2}. There was 1.25 times more flux of liposome as compared with free drug.

DISCUSSION

The regression coefficient (R^2 = 0.999) suggest that the calibration curve of this method is linear within the range, as there was no peak in placebo but peak in the sample at 217 nm. This method is specific to the drug. The percentage drug recovery is within (98–102%), this indicates the method is accurate. The % RSD calculation six sample of of 100% concentration was found to be <2% which indicates this method is précised. The p value obtained by analyzing % drug recovery at three wave lengths using single factor ANOVA indicates that this method is robust. This justified that the method will reproduce accurate, précised and replicable result as it is validated.

Ethanol injection method is poor in drug entrapment efficiency which could be due to large size of vesicles. Factors affecting liposomal particles study showed that ratio of surfactant to cholesterol affects particle size of liposome.[9] Drug entrapment efficiency (65%) was more than obtained by Du and Stanno *et al*.[13,16] Assuming the zeta potential and size of the particle as a key factor to characterize the liposome, so the zeta potential of the sample was measured and found to between −13.7 and −33.75 mV, which suggest that diameter of sample was found to be 73 and 1573 nm [Table 3]. The value of PDI is <1 [Table 3] which suggest the particles are dispersed in the system.

The vesicles of liposome under microscope [Figure 2]. The resultant zeta potential and diameter suggests that the liposomal suspension had sufficient repulsive force to attain better physical colloidal stability and formed multi laminar vesicles. The result of Flux calculation of liposome and free drug in 0.1N HCl indicated that more bioavailable dose can be attained with

Table 3: Zeta-sizer (Malvern Zs) analysis summary of optimized batch (*n*=3)

S. no.	Sample	Size (nm)±Std. dev	*PDI	Mean Zeta potential (mv)±std. dev. (*n*=12)	Zeta Potential (mv) range
1	Optimized formulation_1	985.5±112.6	0.58	−21.9±7.07	−13.7–−33.75
2	Optimized formulation_2	1152±100.8	0.84	−28.2±5.55	
3	Optimized formulation_3	1431±142.4	0.58	−18.4±4.70	

* Polydispersity Index

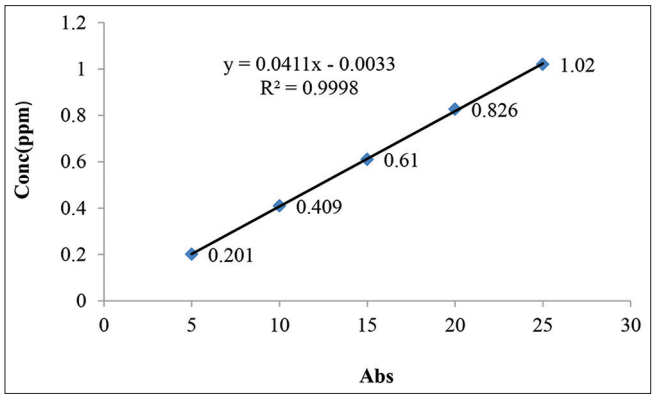

Figure 5: Calibration curve of Fexofenadine in 0.01N HCL

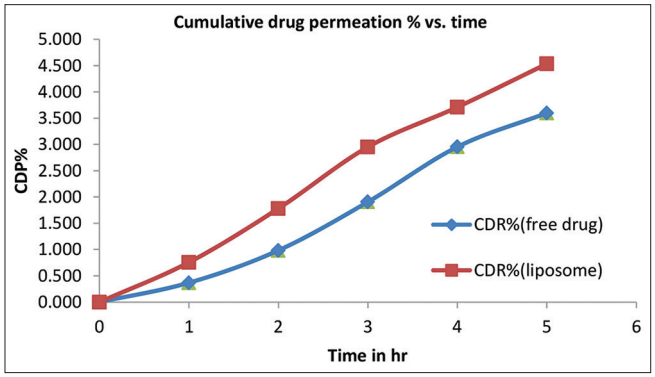

Figure 6: Cumulative drug permeation percentage

liposomal drug delivery than conventional oral dosage form. It suggests that we can reduce the dose to attain same plasma drug concentration if we switch current oral tablet dosage form (120 mg) of fexofenadine to liposomal dosage form [Figure 6] due to better permeation of liposome than drug in solution.

CONCLUSION

This study suggests that BCS class III drug are having permeation rate limiting as a factor of bioavailability. Although it may not affect in therapy for drug having wide range of therapeutic window like fexofenadine, we can have therapeutic effect with fewer doses by increasing permeation or drug absorption by shifting dosage form to liposomes.

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