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Probilosomes: A Novel Bile Salt Containing Nanocarrier for Enhancing Oral Bioavailability

Haritha P, Lakshmi PK*
Department of Pharmaceutics, G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad, Telangana, INDIA.

ABSTRACT

Objective: Probilosomes vesicular drug delivery system was developed to prevent acid degradation of the drug and to improve its oral bioavailability by resisting the drug release in acidic pH of the stomach. **Method:** Thin-film hydration technique was adopted to prepare probilosomes using soybean phosphatidylcholine (lipoid S100) bile salt (Sodium Deoxycholate) and mannitol as a carrier. The formulations were optimized using (3^2) general full factorial design. **Results:** The prepared probilosomal formulations were evaluated for entrapment efficiency, drug content and *in vitro* dissolution studies. Based on the entrapment efficiency values and sustained drug release (PB9) formulation is optimized and further evaluated for surface morphology (SEM), particle size, polydispersity index, zeta potential and *ex vivo* studies. The optimized formulation (PB 9) showed particle size, polydispersity index (PDI) of 76.4 nm, 0.45 and zeta potential of -9.17 mV respectively. *In vitro* dissolution studies showed less than 20% of drug release at pH 1.2 and sustained release in pH 6.8 phosphate buffer up to 8 hr. The *ex vivo* studies indicated a 2 fold increase in the oral bioavailability of the probilosomal formulation compared to pure drug. **Conclusion:** Rosuvastatin calcium probilosomes were successfully prepared to resist the drug release in stomach pH and prevent the acid degradation of the drug. *Ex vivo* studies showed increased oral bioavailability of the probilosomal formulations compared to pure drug. Hence, these formulations can be used as an alternative to conventional enteric dosage forms.

Key words: Bioavailability, Bile salt, Phospholipid, Probilosomes, Rosuvastatin.

Correspondence

Prof. Dr. Lakshmi PK. M.Pharm, Ph.D.
Department of Pharmaceutics, G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad-500028, Telangana, INDIA.
Phone no: +91 9000044452
Email: drlakshmisuresh@gmail.com

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INTRODUCTION

Bilosomes are bile salt incorporated liposomes or niosomes with or without cholesterol in them. Bilosomes are physically and chemically more stable when compared to conventional vesicles (liposomes and niosomes) because bile salts can resist the harsh GIT environment and protect the entrapped drug, unlike conventional vesicles which get degraded in the GIT and may result in the spillage of the drug from the vesicles.[1] Either lipophilic or hydrophilic drugs can be entrapped into the bilosomes. Bile salts-containing vesicles can improve the oral bioavailability of the enclosed drugs as they can act as absorption and penetration enhancers.[1,2] Probilosomes are free-flowing, dry powder formulations consisting of lipids or surfactants, drug and carriers like mannitol, maltodextrin, etc., which, upon hydration disperse to form bilosomal vesicles. Probilosomes can also overcome the problems related to physical stability as they are easy to store, transport, distribute and administer. Rosuvastatin calcium is an anti-hypertensive drug and it belongs to BCS class II was selected as a model drug which is having acid degradation in the stomach and low oral bioavailability (less than 20 %).

MATERIALS AND METHODS

Materials

Rosuvastatin calcium was obtained from Cayman Chemicals, Lipoid S100 was supplied by Lipoid (Germany), Sodium deoxycholate (SDC) was purchased from Sigma Aldrich (India) Mannitol was obtained from Signet Chemical Corporation Private Limited (Mumbai) Ethanol and Dimethyl sulfoxide was obtained from S.D. fine chemicals Limited (India). Male Sprague-Dawley rats were utilized with the approval granted by the Institutional Ethics Committee (IAEC) in G. Pulla Reddy College of Pharmacy Registration Number 320/CPCSEA and student ID number is GPRCP/IAEC/23/19/02/PCE/AE- 4.

Methods

Preparation of probilosomes

Thin-film deposition on carrier method was used for the preparation of probilosomal powder. Weighted amounts of soybean phosphatidyl choline (lipoid S100), bile salt (SDC) and drug were dissolved in 10 ml of ethanol/dimethyl sulfoxide (9/1 v/v) and bath sonicated for 10 min. Then the organic solution was poured on to the carrier (spray-dried mannitol) in a 50 ml round bottom flask connected to a rotary evaporator and subjected to evaporation at 45°C temperature at 100 rpm for about 1 hr. After ensuring the complete drying of the solvent, resultant powder form is further left overnight in vacuum to remove any traces of solvent.[3]

Experimental design

A general full factorial design was employed to optimize the probilosomal formulation Table 2. Factorial design is an effective technique to indicate the relative significance of several variables and their interactions.[4] A 3^2 randomized general full factorial design was utilized in this study. Two factors were assessed each at three levels and formulation trials were carried out with nine possible combinations. Quantity of lipid and quantity of bile salt were taken as the independent variables and % entrapment efficiency is selected as the dependent variable. Table 1 indicates the coded values of independent variables selected based on preliminary trials.

Probilosomes Characterization

Drug-excipient compatibility study by FTIR

The IR spectrum of rosuvastatin calcium, lipoid S100, bile salt and mannitol which were studied using Fourier transform Infra-red spectroscopy (FTIR 8400s, Shimadzu, Japan). By using potassium bromide (KBr) disks FTIR spectra were recorded and analyzed in the range of 4,000-400 cm^{-1}.

Entrapment efficiency

Entrapment efficiency was assessed by using an ultracentrifuge. 100 mg probilosomal powder was reconstituted with 6.8 pH phosphate buffer and transferred into Eppendorf tubes and centrifuged at 8000 rpm for 1 hr at 4°C, where the rosuvastatin calcium loaded bilosomes will settle at the bottom. Then supernatant containing the free rosuvastatin calcium was diluted and analyzed in a UV visible spectrophotometer. (%) EE calculated by using the formula.

$$\text{Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{amount of drug detected in supernatant}}{\text{Total amount drug added}} \times 100$$

Drug content

100 mg of probilosomes formulation was dissolved in 10 ml acetonitrile and sonicated for about 10 mins using bath sonicator. The clear solution after suitable dilution with 6.8 phosphate buffer solution was measured by UV Visible spectrophotometer at 242 nm and the drug content was calculated by the formula.[5]

$$\text{Drug content} = 100 \times \frac{\text{Weight of the drug in the probilosomes}}{\text{Total weight of the probilosomes powder}}$$

Measurement of particle size and zeta potential

The formulation was diluted with water before the measurement to make certain that the light scattering intensity is within the instrument's sensitivity range. The particle size, PDI and zeta potential were measured at 25°C using the light-scattering by Malvern Zetasizer.

In vitro dissolution studies

The *in vitro* drug release studies for the optimized probilosomal formulation were conducted in USP Type II dissolution apparatus. The paddle speed was adjusted to 50 rpm and the temperature of media was kept at 37±0.2°C. Probilosomal formulation equivalent to 5 mg of rosuvastatin calcium filled in a dialysis bag (Molecular weight cut off 12,000-14,000 Dalton) after hydrating with 1 ml distilled water and tied to the paddle. The dissolution study was carried out in 900 ml of 0.1N HCl, for 2 hrs later in 6.8 pH phosphate buffer, for about 8 hrs. Samples were collected at predetermined time points, filtered using Whatman filter paper and analyzed in UV Visible spectrophotometer. The percentage of drug release from the formulation was estimated.[6]

Release kinetics

The release patterns and mechanisms are described by various other linear and non-linear kinetic models that are dependent on the curve fitting procedure. The dissolution profile of PB9 formulation was subjected to Zero-order, First-order, Higuchi and Korsmeyer-Peppas models to find out the kinetic modeling of drug release.

Ex vivo absorption studies

The protocol of the experimental study was approved by the IAEC. Male Sprague-Dawley rats weighing about 200 g were taken and euthanized.

Preparation of intestinal sacs

The animals were housed under normal 12 hr light and dark cycle and optimum temperature with water freely available. The animals were kept fasted for 8 hrs and then sacrificed an insertion was made midline of the abdomen to remove the intestine. The isolated intestine was then washed with an ice-cold oxygenated Krebs ringer solution (KRS). The washed intestine was divided into approximately 6 cm long sacs. The one end of the sacs was tied using braided suture silk, filled with drug solution from the other end through a blunt syringe and then tied. The sacs were immersed in a 100 ml beaker containing 100 ml KRS and the temperature was maintained at 37C with adequate aeration. From outside of the sac 2 ml of samples were withdrawn for a time period of 1 hr and replaced with fresh Krebs ringer solution at different time intervals. The drug content in the samples was analyzed by UV visible spectrophotometer at 242 nm. Rosuvastatin calcium apparent permeability coefficient (P_{app}) was calculated using the equation:

$$P_{app} = (dQ/dt) \times (1/AC_0)$$

Where; P_{app} (cm/sec) is the apparent permeability coefficient, dQ/dt (µg/sec) is the amount of drug transported across the membrane per unit of time, which is obtained from the slope of the linear portion of plot time versus concentration and considered as permeation flux. A (cm^2) is the intestinal sacs surface area accessible for drug permeation and C_0 (µg/ml) is the initial amount of the drug inside the sacs.[7]

Stability studies

Stability studies were carried out by keeping the optimized formulations in amber-colored glass containers, they were in desiccators at room temperature and refrigerator temperature (4±2°C). These studies were performed up to 30 days and at particular time intervals formulation was analyzed for physical appearance (color) and % entrapment efficiency, of bilosomes.[8]

RESULTS

Drug-excipient compatibility studies by FTIR

Compatibility of rosuvastatin calcium and excipients was studied by FTIR. The pure drug showed characteristic peaks at 2970.17 indicating N-H stretching, 2873.74 representing CH_3 symmetric stretch, 2376.14 C=N stretching, 1068.49 C-F stretching 516.89 indicating C=C of benzene ring. The peaks seen in the pure drug were comparatively the same when compared with the optimized formulation spectra, indicating no drug-excipients interaction. Figure 1

Entrapment efficiency

The results showed lipoid S100 in the range of 100-200 mg and bile salt in the range of 25-50 mg showed good entrapment efficiency and the entrapment efficiency decreased on further increase of lipid and bile salt concentrations to 300 and 75 mg. The optimized formulation showed the highest entrapment efficiency of about 63.3%.

Drug content

The content of the drug ranged from 94.24% to 98.23%. PB9 formulations were found to have maximum drug content of 94.92% which indicates that efficient loading into the vesicles and uniform distribution of drug throughout the powder.

SEM

The surface morphology of probilosomal powder was examined using SEM analysis. Figure 2

Particle size and Zeta potential

The size of the bilosomal vesicles generated from probilosomes after reconstituted in water. The mean size, polydispersity index of (PB9) formulation was found to be 70.39 nm and 0.405 respectively.

Zeta potential of the optimized probilosomal formulations (PB9) is (-9.17 mV) negative charged due to the presence of the anionic sodium deoxycholate. Figure 3 and Figure 4

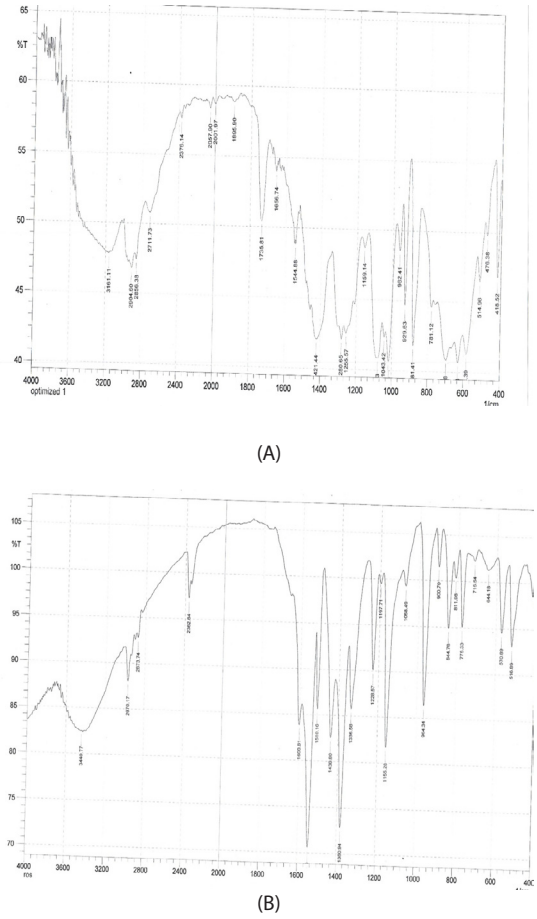
Analysis of results by full factorial design

Factorial design investigates the effect of different independent variables on the prepared probilosomes. The data were plotted on the main effect plots to assess the relationship between the dependent and independent variables. The obtained results (% entrapment efficiency data) response was analyzed by Minitab 18 English software. Figure 5

The magnitude of the inter-relationship effect is high as the slope of the line is steeper. Thus, the inter-relationship effect of lipid is high compared to bile salt. It can be concluded that factor A (quantity of lipid) has more impact on % entrapment efficiency.

In vitro dissolution studies

In vitro release studies were performed for the optimized probilosomal powder (PB9). The percentage release of drug from formulation was



(A)

(B)

Figure 1: (A) FTIR spectra of the pure drug and (B) FTIR spectra of optimized formulation (PB9).

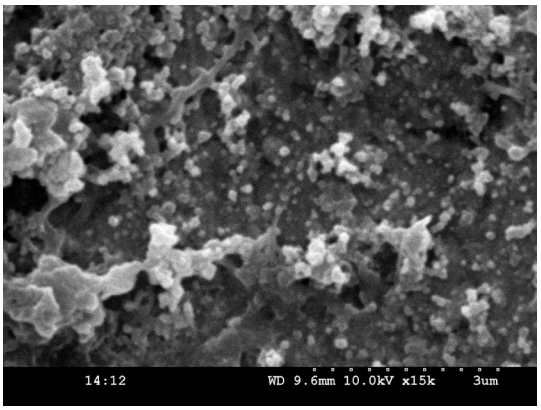


Figure 2: SEM Images of optimized formulation.

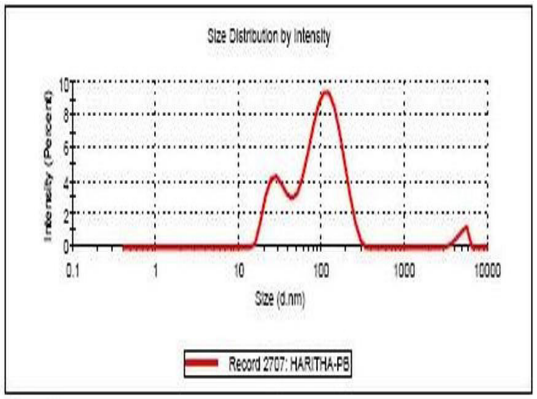


Figure 3: Particle size and PDI PB9 formulation.

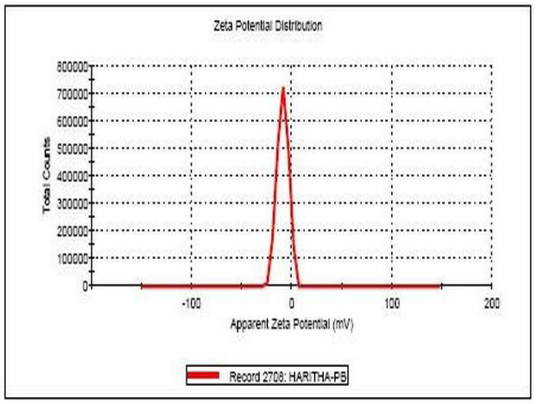


Figure 4: Zeta potential of PB9 formulation.

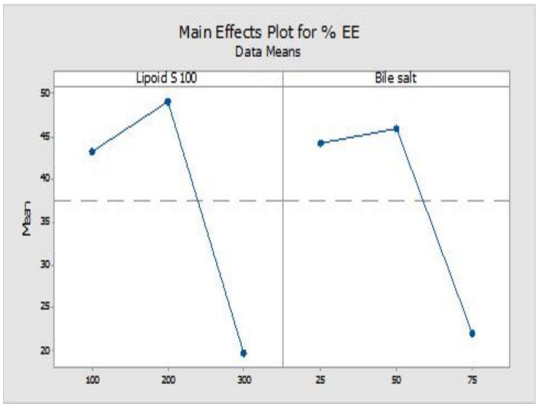


Figure 5: Main effect plot for % entrapment efficiency.

calculated. To ensure the stability of the bilosomes during the passage through the gastrointestinal tract, the leakage of the entrapped rosuvastatin calcium from the probilosomal formulations (PB9) was investigated *in vitro*, through the dissolution in 0.1 N HCl media. The optimized formulation showed (18.48 %) after 2 hr in 0.1 N HCl (pH 1.2) and (83.48 %) drug release after 10 hrs in 6.8 pH phosphate buffer. Therefore, the probilosomes formulation (PB9) rosuvastatin calcium encapsulated is more stable in the acidic pH. The probilosomal formulated showed a sustained drug release mechanism.[3]

Model dependent kinetics of optimized formulations

The optimized probilosomal formulation followed first-order drug release kinetics with the Higuchi drug release mechanism.[1] The value of release component "*n*" indicates anomalous diffusion release mechanism. The release process involves the penetration of water into the powder followed by the dissolution of the carrier, lipid and diffusion of the drug dissolved in the matrix. Table 3

Ex vivo studies – intestinal absorption rosuvastatin

Ex vivo study was performed using a non-everted rat intestine. The surface area A (cm^2) of intestinal sac assuming to have a cylindrical shape with a length 5 cm and radius 0.3 cm was calculated as 9.9852 cm^2 per sac.[7]

The apparent permeability coefficient (P_{app}) of optimized formulation was 2 fold higher than the pure drug indicating an increased oral bioavailability of the rosuvastatin containing probilosomes compared to pure drug solution reasons behind the improved bioavailability may be due to mucolytic and penetration enhancing effect of sodium deoxycholate which can loosen the tight junctions in the epithelial lining of the intestinal membrane by binding to Ca^+ channels might have contributed in enhanced permeation of probilosomes.[9-11]

Stability studies

The stability studies were conducted for one month at room temperature and refrigerator (4±2°C). At defined time period, the probilosomal formulation was evaluated for physical appearance and encapsulation efficiency, not much change was observed in the physical appearance and % encapsulation efficiency which indicates the stability of probilosome

Table 1: Selection of independent variables for a (3^2) full factorial design.

Factors (Independent variable)	Levels		
	-1	0	+1
Quantity of lipid (Factor A)	100 mg	200 mg	300 mg
Quantity of Bile salt (Factor B)	25 mg	50 mg	75 mg

Table 2: Full Factorial design (3^2) runs formulae

Materials	PB1	PB2	PB3	PB4	PB5	PB6	PB7	PB8	PB9
Drug (mg)	40	40	40	40	40	40	40	40	40
Lipoid S100 (mg)	100	100	300	300	200	200	100	300	200
Bile salt (mg)	75	50	50	75	75	50	25	25	25
Mannitol (mg)	1000	1000	1000	1000	1000	1000	1000	1000	1000
Ethanol (ml)	9	9	9	9	9	9	9	9	9
DMSO (ml)	1	1	1	1	1	1	1	1	1

mg: milligram, ml: milliliter, DMSO: Dimethyl sulfoxide.

Table 3: *In vitro* drug release kinetics of optimized formulation.

Formulation	r^2				*n*	Drug transport mechanism
	Zero order	First-order	Higuchi	Korsmeyer Peppas		
PB9	0.946	0.974	0.966	0.926	0.732	Anomalous transport

Table 4: Apparent permeability (P_{app}) of rosuvastatin calcium through non everted sac.

Applied solutions	P_{app} (cm/sec) x 10^{-6}
Pure drug	1.4±1.23
PB9	3.4±0.47

The values are expressed as their mean ±SD, n=3
cm: centimeter, sec: second

formulation both stored at room temperature and refrigerated temperature.

DISCUSSION

Probilosomes were successfully prepared using thin film deposition on carrier method. The effect of varying amounts of lipid and bile salt on the entrapment efficiency has been studied. It was seen higher amount of lipid or bile salt decreases the encapsulation efficiency reason may be increased concentration of lipid or bile salt may lead to surfactant action on the bilosomal lipid bilayers resulting in destroyed vesicular structure leading to poor encapsulation efficiency of the drug.[8,12] Based on the full factorial experimental design analysis it was found that concentration of lipid is effecting more on % entrapment efficiency than the concentration bile salt. The flow properties of the powder obtained were determined. Sticky formulations were avoided. Based on the entrapment efficiency and flow properties, optimised formulations were chosen for further study. The *in-vitro* dissolution and diffusion of developed formulation showed a sustained drug release profile following first-order kinetics and Higuchi release mechanism. From the values of release component "*n*," it is evident that the formulation has an anomalous diffusion release mechanism. *Ex-vivo* studies performed using non-everted rat intestine studies showed significant enhanced drug absorption. The apparent permeability coefficient (P_{app}) of optimized formulation was 2-fold higher than the pure drug. This shows an increased oral bioavailability of the rosuvastatin containing probilosomes compared to pure drug solution. The reasons could be due to mucolytic and penetration enhancing effect of sodium deoxycholate which can loosen the tight junctions in the epithelial lining of the intestinal membrane by binding to Ca^{+} channels might have contributed in enhanced permeation of probilosomes.[9-11] Thus this study showed that probilosomes can increase the absorption of the drug due to the effect of bile salts and it can be a useful formulation for acid labile drugs to protect the drug from harsh GI environment. Table 4

CONCLUSION

Probilosomes vesicular drug delivery system was prepared in order to prevent acid degradation of the drug and to improve its oral bioavailability by resisting the drug release in the stomach. Rosuvastatin calcium which is having instability in acidic media and low oral bioavailability was selected as a model drug and successfully formulated as probilosomal powder. Probilosomes were prepared using three different concentrations of lipid and bile salt and their effect on % entrapment efficiency was assessed using full factorial design. The results indicated that probilosomes can increase the oral bioavailability of less soluble drugs and also can be used as a substitute for conventional enteric-coated dosage forms.

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CONFLICT OF INTEREST

The authors declare no conflict of interets.

ABBREVIATIONS

None.

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