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PEGylated liposome containing sildenafil for the treatment of hypertension

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ABSTRACT

The purpose of this study was to prepare PEGylated liposomes containing sildenafil for the treatment of pulmonary arterial hypertension. PEGylated liposomes were prepared by thin film hydration method by varying the concentration of lipids. The prepared liposomes were characterized for the particle size, PDI, zeta potential, % entrapment efficiency and *in-vitro* release study. The optimized formulation exhibits a particle size of 104.27±1.4 nm, PDI of 0.449±0.02, zeta potential of -42.9±0.7 along with the maximum encapsulation of drug i.e. 87.30±2.6. Optimized formulation showed % cumulative drug release of 85.38±0.26 in 48 hr. From the study it can be concluded that the PEGylated liposomes containing sildenafil for the treatment of pulmonary arterial hypertension provides a sustained release of drug and further studies are required to confirm their efficacy at clinical level.

Keywords: Sildenafil; PEGylated liposomes; Pulmonary arterial hypertension; % entrapment efficiency

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INTRODUCTION

Pulmonary arterial hypertension (PAH) is a hemodynamic and pathophysiological condition of the lungs exhibiting an elevated mean arterial pressure and vascular resistance[1]. The available treatment for PAH including endothelin receptor antagonist, calcium antagonist, anticoagulants, prostacyclin or inhaled nitrous oxide etc. have several associated side effects such as nausea, vomiting, flushing, liver damage etc[2]. USFDA have approved sildenafil for the treatment of PAH which is a Phosphodiesterase type 5inhibitor improves blood flow distribution areas and reduces resistance at blood vessels[3].

Liposomes or lipid vesicles are colloidal particle composed of lipid molecules majorly having at least one enclosed lipid bilayer. Liposomes have been considered as an attractive drug delivery system for pulmonary application. In spite of these facts, a major drawback corresponding to liposomal formulation is its rapid clearance from the blood due to adherence of plasma protein on phospholipids. To overcome this issue, surface of the drug bearing particle is treated with PEG of different molecular weight and designated as PEGylated liposome which offers release of drug in controlled manner[4, 5].

In the present study PEGylated liposomes were prepared and characterized for the particle size, PDI, zeta potential, % entrapment efficiency and *in-vitro* release study.

MATERIAL AND METHODS

Sildenafil was purchased from Cadila Pharmaceutical, Ahmedabad, India. Leciva S-90 was obtained as a gift sample from VAV life sciences Pvt Ltd Mumbai, Maharashtra, India. Cholesterol was purchased from Sigma-Aldrich Chemical, USA. Distilled water (HPLC grade) was purchased from Merck specialties Pvt. All other chemicals used were of analytical grade.

Preparation of PEGylated liposomes

PEGylated liposomes were prepared by thin film hydration method by varying the concentration of lipids as shown in Table 1. To prepared liposomes firstly the drug and lipid were weighed and were taken in round bottom flask and dissolved in 5ml of chloroform. Then the RBF was attached with the rotary evaporator with the maintained water bath temperature at 40°C with 40 rpm. Further organic solvent was evaporated to form the thin layers of lipids under reduced pressure. Afterwards for the complete removal of organic solvent and drying of the lipid film RBF was placed in desicator for 2 hr. Then the lipid film was hydrated with the 20ml of distilled water and Tween 80 on rotary evaporator with water bath temperature maintained at 60°C with 100 rpm untill the lipid film gets completely hydrated to form the liposomal suspension. Then the prepared liposomal suspension was subjected to probe sonication for two cycles of 10 mins so as to reduce the size of the vesicles and then kept in refrigerator [5,6].

Table 1 Composition of the PEGylated liposome loaded with sildenafil

Material	SL-1	SL-2	SL-3	SL-4	SL-5	SL-6
Sildenafil (mg)	10	10	10	10	10	10
Leciva S-90(mg)	180	140	180	140	180	140
Cholesterol (mg)	10	20	10	20	10	20
MPEG-DSPE$_{2000}$(mg)	-	-	25	25	50	50
Chloroform (ml)	5	5	5	5	5	5
Tween$_{80}$(%w/w)	6	6	6	6	6	6
Distilled water (ml)	20	20	20	20	20	20

Characterization of PEGylated liposomes

Particle size, PDI, Zeta potential

Zeta potential, PDI and particle size are very crucial parameters for the any developed formulation. Malvern zeta sizer ZS (Malvern Instrument UK) based on the principle of dynamic light scattering (DLS) was used to measure the particle size, zeta potential and PDI of PEGylated liposomes. For the determination of particle size, liposomal suspension was diluted 1 in 10ml with phosphate buffer saline. PDI was determined for evaluation of particle size distribution. Zeta potential is expressed as overall charge that the particle acquires in a particular medium. It was determined with help of Malvern zeta sizer based on the principle of laser dopler velocimetry and phase analysis scattering. Zeta potential above than +30mv and -30mv are considered as more stable[7, 8]. All the measurements were taken in triplicate and expressed in mean ± SD.

% Entrapment efficiency

The entrapment efficiency of sildenafil PEGylated liposome was measured by the ultracentrifuge method. Entrapment efficiency enables to quantify the amount of drug entrapped within a liposome. In an outline, vesicular preparations containing 1% sildenafil were kept overnight at 4°C and centrifuged in a ultracentrifuge (Remi) equipped with TLA-45 rotor at 4°C on 30 000 rpm for 2 h. The concentration of entrapped drug was determined with the help of UV/visible spectrophotometer[9, 10]. The amount of drug entrapped within the colloidal system was determined with the help of given formula-

$$\% \text{ EE} = \frac{amount\ of\ free\ drug}{total\ amount\ of\ drug} \times 100$$

***In-vitro* drug release study**

The drug release from PEGylated liposomes was accomplished with the help of diffusion cell. Prior to work, a cellulose acetate membrane was soaked in distilled water for 24 h, so that it can be effortlessly attached to the donor compartment of the diffusion cell. Further, the diffusion cell was secured with the help of clamp stand and submerged in a receptor compartment containing 100ml of phosphate buffer (pH 6.8) maintained at 37°C. Liposomal formulation containing single dose equivalent to the sildenafil in donor compartment was covered with the help of paraffin so as to avoid evaporation of the solvent. The whole assembly was kept on magnetic stirrer with continuous stirring at 750rpm. 3ml of solution was withdrawn and replaced by same volume of phosphate buffer in the receptor compartment at a specific interval of 0-24h respectively[11]. The withdrawn sample was observed in UV/visible spectrophotometer to determine the drug concentration which was further used to determine the % cumulative drug release and plotted against time[12].

RESULTS AND DISCUSSION

Particle size and their size distribution are the important parameters which describe the quality of liposomal formulation and were determined with the help of Malvern zeta sizer ZS based on the principle of DLS. The result of particle size and PDI was shown in Table 2. All formulations i.e. from SL-1 to SL- 6 showed a mean particle size ranging from 104.27±1.4 to 137.01±3.7 and PDI ranging from 0.254±0.04 to 0.449±0.02. From the obtained results it was observed that a small variation in the concentration of lipids greatly affects the particle size. As observed that at higher concentration of lipid (Leciva S-90) and at lower cholesterol concentration minimum particle size was obtained as in FormulationSL-3 but at lower concentration of leciva S-90 and higher concentration particle size was increased. According to the results, formulation SL-3 was optimized. The stability of the PEGylated liposome was determined by zeta potential using Malvern Zeta sizer ZS based on principle of laser dopler velocimetry and phase analysis scattering. The particles of the liposomal suspension with the value of zeta potential above than +30 and -30mv are considered as most stable. The result of the zeta potential was shown in Table 2. All formulation exhibited the zeta potential ranging from -15.2±0.4 to -42.9±0.7. Negative charge on the surface of the liposomes was due to the presence of tween-80. The results indicate that at higher concentration of leciva S-90 the zeta potential values were away from zero. These findings indicated that stability of the liposomal formulation rely on the concentration of lipid used in formulation. A proper amount of lipid ratio produces accurate result and according to which the formulation SL-3 was optimized.

% Entrapment efficiency

Entrapment of drug molecule in the PEGylated liposomes depends on the total amount of lipid, total amount of drug and drug to the lipids ratio to be added. % entrapment efficiency was determined with the help of centrifugation method and the results were shown in Table 2 and Figure 1. In the absence of MPEG-DSPE$_{2000}$ formulation SL 1 and SL 2 show entrapment of 70.21±2.0 and 68.93±0.8 respectively, whereas formulation SL 3 with low concentration of MPEG-DSPE$_{2000}$ and cholesterol and high concentration of leciva S-90 show entrapment of 87.30±2.6 respectively. In addition formulation SL 4 with low concentration of leciva S-90 and MPEG-DSPE$_{2000}$ and high concentration of lipids show entrapment of 75.32±2.4 respectively whereas formulation SL 5 and SL 6 with increased MPEG-DSPE$_{2000}$ concentration show entrapment of 82.65±1.4 and 54.19±0.9 respectively. Among all formulations SL 3 show maximum entrapment of drug molecules. Result indicates that at higher concentration of leciva S-90 entrapment efficiency of liposomes was increased. Tween 80 helps to provide to solubilizing effect due to which the amount of sildenafil in the inner aqueous phase was increased. At higher concentration of MPEG-

$DSPE_{2000}$ no significant increase in the entrapment was observed because of the increased binding of sildenafil to the lipid occurs. Cholesterol helps in lipid bilayer packing but at higher concentration lipid membrane packed tightly which decreases the entrapment of drug. From the study it was concluded that appropriate amount of lipid is necessary for the higher encapsulation of drug in the PEGylated liposomes. According to the results formulation SL 3 was optimized.

Table 2 Characterization of PEGylated liposome of Sildenafil

characterization	SL-1	SL-2	SL-3	SL-4	SL-5	SL-6
Vesicle size	111.47±2.8	116.38±3.4	104.27±1.4	120.32±1.6	118.39±2.9	137.01±3.7
PDI	0.312±0.07	0.254±0.04	0.449±0.02	0.267±0.03	0.382±0.06	0.297±0.05
Zeta potential	-28.6±0.4	-24.7±0.3	-42.9±0.7	-15.2±0.4	-36.8±0.2	-30.2±0.6
%EE	70.21±2.0	68.93±0.8	87.30±2.6	75.32±2.4	82.65±1.4	60.19±0.9

All data are expressed as mean ± S.D.; n = 3

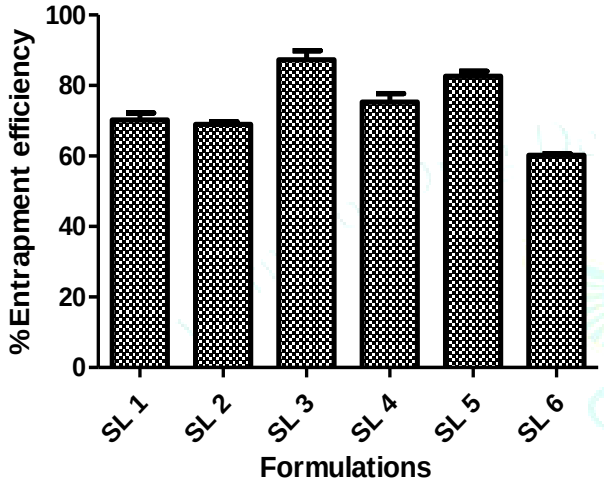

Figure 1: Percentage entrapment efficiency of PEGylated liposomal formulations

In-vitro drug release study

The *in-vitro* release of all the formulations loaded with sildenafil was studied using diffusion cell. The result of *in-vitro* release was depicted in Figure 2. Formulations SL-1 to SL-6 showed 68.23±0.25, 63.96±0.67, 85.38±0.26, 72.65±1.62, 78.14±1.56, 60.19±1.25 drug release in 48 hr respectively. Formulation SL-3 showed maximum release of drug because of high encapsulation of drug in the PEGylated liposome. The release of sildenafil from formulation was rapid for initial 2 h and later on it followed sustained release and when the concentration of leciva S 90 was increased then the release of drug reduces because the drug was entrapped in the phospholipid bilayer membrane and the membrane was stabilized with cholesterol and tween 80 so the drug takes time to get released. Thus a depot effect was achieved for the release of drug from the PEGylated liposome. The formulation SL 6 showed minimum release of drug in 24h because of low encapsulation and presence of high concentration of $MPEG\text{-}DSPE_{2000}$ and cholesterol. From this study, it was concluded that small variations in the concentration of lipids may produces a drastic variation in the release of drug. According to the results, formulation SL-3 showed sustained release of drug and was optimized formulation. This factor was further supported by observation of Shavi et al. 2015; and Dave et al. 2017.

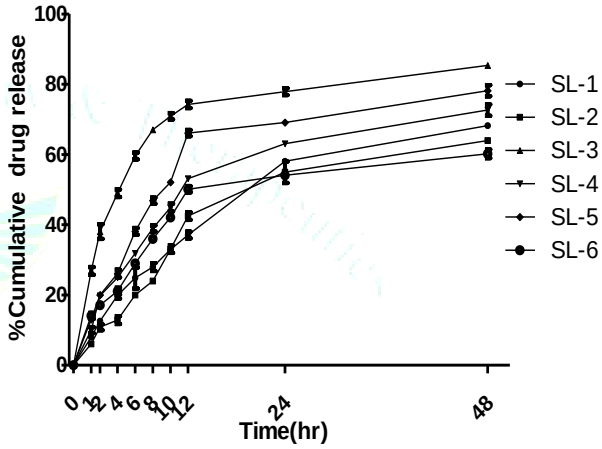

Figure 2: In-*vitro* drug releases of PEGylated liposomal formulations

CONCLUSION

In the present PEGylated liposomes were prepared by thin film hydration method with varying lipid concentration. Result shows a controlled particle size, good zeta potential which indicates that the formulation was stable along with maximum encapsulation of drug in the liposomes. Optimized formulation show a maximum release of drug in 48 hr which show that the PEGylated liposomes provide a sustained release of sildenafil for the treatment of pulmonary arterial hypertension. Further *in-vivo* study and more research work are needed to bring the PEGylated liposomes into its clinical realization.

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REFERENCES

1. Gaine SP, Rubin LJ, Primary pulmonary hypertension. Lancet, 1998; 352:719-25.
2. Dupuis J, Hoeper M M,. Endothelin receptor antagonists in pulmonary arterial hypertension. Eur. Respir. J. 2008; 31:407–415.
3. Ghasemian E, Vatanara A, Rouini MR, Najafabadi AR, Gilani K, Lavasani H, Mohajel N. Inhaled sildenafil nanocomposites:

lung accumulation and pulmonary pharmacokinetics. Pharm Dev Technol, 2015; 1-11.
4. Atyabi F. Dinarvand R, Preparation of PEGylated nano liposomal formulation containing SN-38: in vitro characterization and in vivo biodistribution in mice. Acta Pharmaceutica 2009; 59:133-144.
5. Shavi GV, Reddy MS, Raghavendra R, Dave V, Kushwaha K. PEGylated liposomes of anastrozole for long-term treatment of breast cancer: in-vitro and in-vivo evaluation. J Liposome Res: 2015; 1-9.
6. Maalej CJ, Charcosset C, Fessi H, A new method for liposome preparation using a membrane contactor. J Lipo Res 2011; 21:213–220.
7. Dmitry V, Schaaf P, Mohwald H, Effective embedding of liposomes into polyelectrolyte multilayered films: the relative importance of lipid-polyelectrolyte and inter polyelectrolyte interactions. RSC Adv 2009; 5:1394–1405.
8. Makino K, Yamada T, Kimura M et al, Temperature- and ionic strength-induced conformational changes in the lipid head group region of liposomes as suggested by zeta potential data. Biophy Chem 1991; 41:175–183
9. Betageri GV, Parsons DL Drug encapsulation and release from multilamellar and unilamellar liposomes.Int J Pharm 1992; 81:235–241.
10. Dave V, Sharma S, Yadav RB, Agarwal U. Herbal liposome for the topical delivery of ketoconazole for the effective treatment of seborrheic dermatitis. Appl Nanosci. 2017; 8:973-87.
11. Y Er, Barnes TJ, Fornasiero D, Fornasiero CA, The encapsulation and release of guanosine from PEGylated liposomes. J Liposome Res 2009; 19:29–36.
12. Sezer AD, Bas AL, Akbuga J, Encapsulation of enrofloxacin in liposomes: preparation and *in-vitro* characterization. J Liposome Res 2004; 14(1&2):77–86.