International Journal of Medicine and Pharmaceutical Research
Home Page: https://pharmaresearchlibrary.org/journals/index.php/ijmpr
CODEN (USA): IJCPNH | ISSN: 2321-2624 | Publisher: Pharma Research Library
DOI: https://doi.org/10.30904/j.ijmpr.2025.4793
Int. J. Med. Pharm. Res., 2025, 13(1): 41-47

Preparation and Evaluation of Sildenafil Loaded with Pegylated Liposomes used for Hypertension

Sarvepalli Revathi*[1], Y. Bhargavi[2], K. Sai Sreya[2], B. Nithin[2], G. Sai Sarath Kumar[2], Y. Naga Lakshmaiah[2], Y. Prapurna Chandra[3]

[1]Associate Professor, Department of Pharmaceutics, Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore-524346, A.P., India.
[2]B.Pharm 4th Year, Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore-524346, A.P, India
[3]Professor & Principal, Department of Pharmacology, Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore-524346 A.P., India.

A B S T R A C T

PEGylated liposomes were successfully synthesized using the thin film hydration method to enhance the pharmacokinetic profile of sildenafil. The developed liposomal formulations exhibited optimal physicochemical properties, including desirable particle size, low polydispersity index (PDI), favorable zeta potential, and high encapsulation efficiency. Among the various formulations, SL-3 demonstrated superior performance, showing enhanced vasorelaxation activity and a significant reduction in mean systolic arterial pressure compared to conventional sildenafil and the marketed formulation Revetio®. The PEGylated liposomes provided sustained drug release, improved bioavailability, reduced dosing frequency, and enhanced stability, contributing to better patient compliance with minimal adverse effects. The nanoscale size of these liposomes supports their suitability for parenteral administration and prolonged therapeutic action. While the results are promising, further investigations are warranted to validate their efficacy under various physiological and pathological conditions.

Keywords: PEGylated liposomes, sildenafil, thin film hydration, encapsulation efficiency, sustained release, bioavailability, vasorelaxation.

A R T I C L E I N F O

***Corresponding Author**
Sarvepalli Revathi
Associate Professor, Department of Pharmaceutics
Ratnam Institute of Pharmacy,
SPSR Nellore-524346, A.P., India.

Article History:
Received : 11 Mar 2025
Revised : 20 Mar 2025
Accepted : 05 April 2025
Published : 21 April 2025

Citation: *Sarvepalli Revathi, et al Preparation and Evaluation of Sildenafil Loaded with Pegylated Liposomes used for Hypertension. Int. J. Med. Pharm. Res., 2025, 13(1):41-47.*

Contents

1. Introduction

Sildenafil was the first oral medication approved by the U.S. Food and Drug Administration (FDA) for the treatment of erectile dysfunction (ED) [1,2]. Initially developed as a potential therapy for hypertension and angina pectoris, it failed to demonstrate efficacy for these indications during Phase I clinical trials. However, it produced an unexpected pharmacological effect significant penile erection in trial participants [1,3]. This serendipitous outcome led to Pfizer patenting sildenafil in 1996, followed by its FDA approval for ED treatment in 1998. Since then, various additional therapeutic applications of sildenafil have been identified [4]. Pharmacologically, sildenafil acts as a potent and selective inhibitor of phosphodiesterase type 5 (PDE5) [5,6]. Although multiple phosphodiesterase isoforms exist, only PDE5, PDE6, and PDE9 show selectivity for cyclic guanosine monophosphate (cGMP) over cyclic adenosine monophosphate (cAMP) [7,8]. PDE5 catalyzes the hydrolysis of cGMP into its inactive form, 5′-guanosine monophosphate (GMP), thus modulating various physiological functions such as neuroprotection,

antinociception, synaptic plasticity, calcium regulation, and vasodilation [9,10]. cGMP exerts these effects by activating ion channels, cGMP-dependent protein kinases, or by interacting with specific phosphodiesterases [9]. Its production is stimulated by nitric oxide (NO), which, together with cGMP, forms a critical signalling pathway operating through autocrine, paracrine, and potentially endocrine mechanisms. Dysregulation of the NO/cGMP /PDE5 pathway has been implicated in several conditions, including neurological disorders, pulmonary arterial hypertension, cardiomyopathy, cancer, ED, and lower urinary tract syndrome [8,11–14].

In addition to its primary mechanism of action, sildenafil may exert several supplementary therapeutic effects through alternative biological pathways. Notably, it has shown potential in the treatment of neurodegenerative disorders by activating peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α), following cGMP accumulation. This activation promotes mitochondrial biogenesis [15,16], enhances the expression of antioxidant enzymes [17], and reduces the expression of β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) [18].

Sildenafil also demonstrates anti-inflammatory and neuroprotective properties, likely mediated via the AMP-activated protein kinase (AMPK)/inhibitor of nuclear factor kappa-B alpha (IκBα)/nuclear factor kappa B (NF-κB) signaling pathway [19]. Additionally, endothelial nitric oxide synthase (eNOS) plays a role in sildenafil's neuroprotective effects by facilitating AMPK activation [19,20]. Evidence also suggests that phosphodiesterase type 5 (PDE5) inhibitors can alleviate chronic pain by preventing the downregulation of angiopoietin-1 expression a key regulator of vascular stability and neurite outgrowth in cultured dorsal root ganglion neurons [21,22].

Furthermore, sildenafil may enhance the efficacy of chemotherapeutic agents by increasing tumor cell sensitivity to cytotoxic stress. This chemosensitizing effect involves the promotion of apoptosis through down regulation of B-cell lymphoma-extra large (Bcl-xL) and Fas-associated phosphatase-1 (FAP-1), increased production of reactive oxygen species (ROS), and the upregulation of caspase-3, -8, and -9 activities [23–25].

2. Materials and Methods

Sildenafil was purchased from Cadila Pharmaceutical, Ahmedabad, India. Leciva S- 90 and Leciva DSPC 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 Liposome

PEGylated liposomes were prepared using thin film hydration method, where, a known amount of lipids such as leciva DSPC, leciva S-90, cholesterol and drug (sildenafil) were weighed in different lipid molar ratio as given in Table3.1. The weighed constituents were taken in 100ml of round bottom flask (RBF) and 5ml of chloroform was added to it respectively. The RBF was attached to rotary evaporator with a water bath maintained at temperature of 40°C at 50rpm. Organic solvent was further evaporated under reduced pressure to form a thin layer of lipids on the wall of RBF (Maalej et al. 2011; Shavi et al. 2015)[2,8]. Afterwards, the RBF was placed in dessicator for 24h to assure the complete removal of solvent and complete drying of lipid film.

The lipid film was hydrated with 20ml of distilled water along with tween by attaching the RBF to the rotary evaporator under maintained rotation speed of 100rpm and water bath temperature at 60° C until the lipid film gets completely hydrated to form the liposomal suspension. Further, to reduce the size of liposomal vesicles the mixture was probe sonicated for two cycles of 10mins each and kept in refrigerator for 2h. The liposomal suspension was further centrifuged at 1000 rpm to remove the un-entrapped drug and the supernatant was replaced by the addition of the phosphate buffer saline (pH 7.4). Liposomal pellets were separated and washed with phosphate buffer saline (pH 7.4) and re-suspended into the de ionized water containing 50mg/ml trehalose (cryoprotectant) before pre-freezing process of lyophilisation. Afterwards, the frozen sample of the PEGylated liposomes was lyophilized by using Labconco lyophilizer at -80°C temperature and 0.018 mBar vacuum pressures till complete drying was achieved. The dried PEGylated liposomes were immediately collected and then stored in the dried vials till the further usage[9].

Characterization of PEGylated Liposome Particle Size, PDI, Zeta Potential Analysis

Zeta potential, PDI and particle size are very crucial parameters for 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 10-11. All the measurements were taken in triplicate and expressed in mean± SD.

Surface morphology of vesicle

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM): The surface morphology of the lyophilized sample was performed with the help of SEM (EVO 18, Zeiss, Germany). Prior to analysis, the sample was placed on double sided carbon adhesive tape, with gold uniform sputtering and was analyzed at different magnification power (Benoy et al. 2009). The morphology and size of prepared ethanolic liposome were also evaluated using TEM operated at 200kV and 9000x magnification. In brief, 1ml of the formulation taken in eppendorf tube was diluted 10 times by deionized water. Further, on a coated

copper grid small amount of sample was dried after staining with 2% w/v phosphotungustic acid for 30 s. Each sample was prepared and viewed randomly using calibrated microscopic magnification 12.

Attenuated Total Reflection- Fourier Transform Infra Red Spectroscopy (ATR- FTIR)

IR Spectra of pure drug, leciva- DSPC, leciva S-90, optimized formulation and physical mixture was performed with the help of ATR–FTIR spectra at room temperature using Bruker EQUINOX 55FTIR spectrophotometer equipped with liquid nitrogen cooled mercury cadmium telluride (MCT) detector with nominal resolution of 2 cm-1. Diamond an internal reflector, placed at an incidence angle of 45°, scans 32 times and gave 21 resolutions from a single internal reflection. An advanced ATR correction was applied to all spectra recorded in region of 4000-400 cm-1 and peak fitting was performed with the help of Opus software 13.

RAMAN Spectroscopy

Raman spectroscopy aids to deliver the information on rotational, vibrational and other low- frequency modes in the given system. Raman was carried out using thermo-scientific instrument (DxRxi), equipped with a software OMINICxi-analysis. The 532nm laser beam was used to collect the spectra of sildenafil loaded PEGylated liposomal formulation with the laser power of 5-100mW in the range of 125-4000cm-1. 14

Differential Scanning Calorimetry (DSC)

DSC was accomplished with NETEZCH DSC 204 F1 phoenix in DSC chamber. The instrument comprised of a thermal analyzer, a calorimeter, a flow controller and operating software. In a nutshell, pure drug (sildenafil), leciva-DSPC, leciva S-90, cholesterol and optimized formulation were weighed individually in an aluminium pan and sealed with an aluminium lid respectively. Afterwards, the pan was placed in the DSC and heated from 20-350°C at a heating rate of 50°C/min in nitrogen atmosphere. The scan was observed and plotted representing heat flow (w/g) on the Y-axis and temperature on the X-axis 15.

% 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 16-17. The amount of drug entrapped within the colloidal system was determined with the help of given formula:

% EE = [Amount of entrapped drug/Total amount of drug added] x100

In - vitro Drug Release

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 a nd 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 750 rpm. 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 (Y Er et al. 2009). 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[18].

3. Results and Discussion

Particle Size, PDI and Zeta Potential

Particle size and their size distribution are important parameters which describe the quality of optimized liposomal formulation. They were determined with the help of Malvern zeta sizer ZS based on the principle of DLS. The result of particle size and PDI is shown in Table 3.3. All formulations i.e. from SL-1 to SL- 6 showed a mean particle size ranging from 91.14±1.4 to 131.09±3.7 and PDI ranging from 0.478±0.02 to 0.624±0.05. From the above study it was observed that variation in lipid concentration greatly affected particle size and their distribution. For instance, formulation SL-6 with higher concentration of leciva DSPC and cholesterol showed greater particle size i.e 131.09±3.7 with the PDI of 0.624±0.05. On contrary, with low concentration of cholesterol and leciva DSPC along with the high concentration of leciva S-90 as in formulation SL-3 showed smaller particle size i.e 91.14±1.4 with narrow PDI of 0.478±0.02. Obtained results indicated that an increase or decrease in the lipid ratio drastically affects the particle size. In addition, formulation SL-3 (lipid ratio of 9:1:0.25) with desired particle size and PDI was considered 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. All formulation exhibited the zeta potential ranging from -16.4±0.6 to -30.9±0.7. Negative charge on the surface of the liposomes was due to the presence of tween-80. In view of abovementioned, it was concluded that in the presence of higher concentration of leciva DSPC as in formulation SL-6 the value of zeta potential was towards zero i.e. -16.4±0.6. But zeta potential of formulation SL-3 with leciva DSPC and cholesterol in lower concentration along with the higher concentration of leciva S-90 was away from zero i.e. -30.9±0.7mv. 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. According to Shavi et al. the drug: lipid ratio affect the size of particle, zeta potential in the liposome formulation and also the stability during storage. Liposome instability occurs through the agglomerate formation during the formulation processing and upon storage [2].

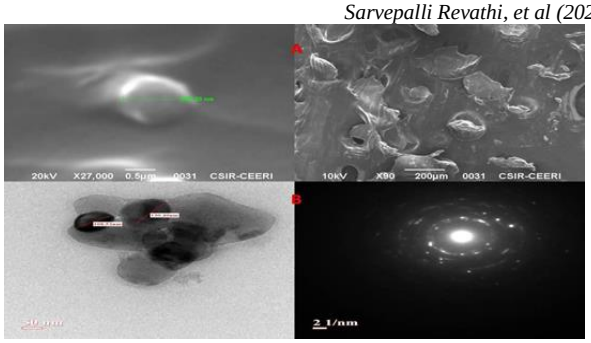

Fig.1 Microscopic morphology of optimized formulation SL-3 shown by [A] SEM [B] TEM

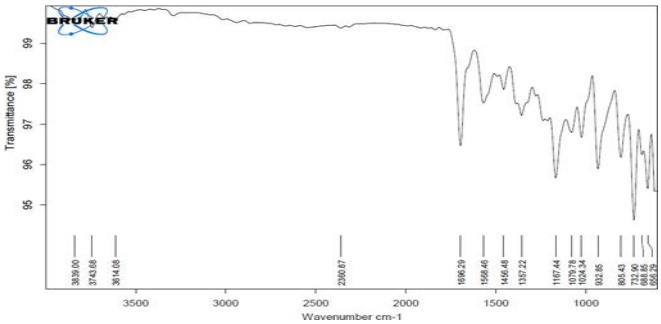

Fig.2 Attenuated Total Reflection- Fourier Ttransform Infra Red Spectroscopy (ATR- FTIR)

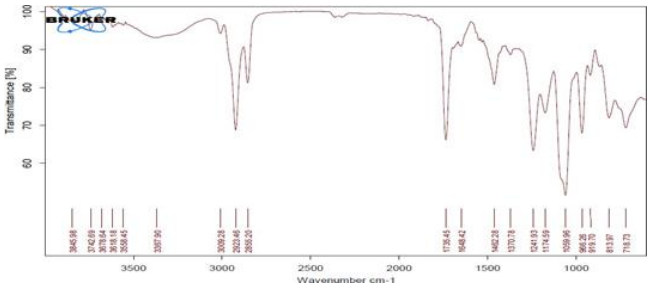

Fig.3 FTIR spectra of Sildenafil

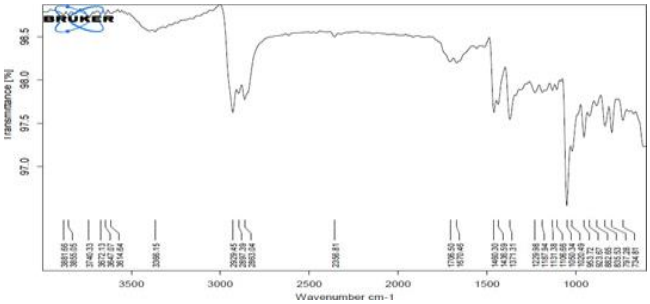

Fig.4 FTIR spectra of Leciva S-90

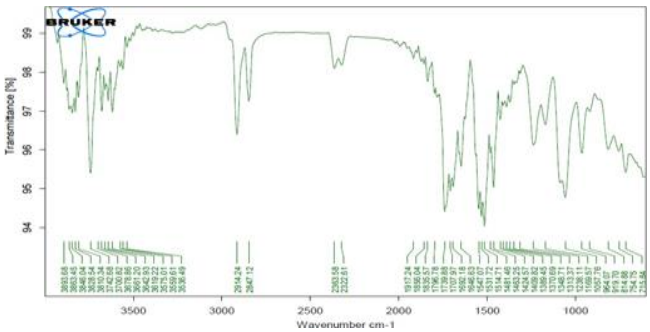

Fig.5 FTIR spectra of Cholesterol

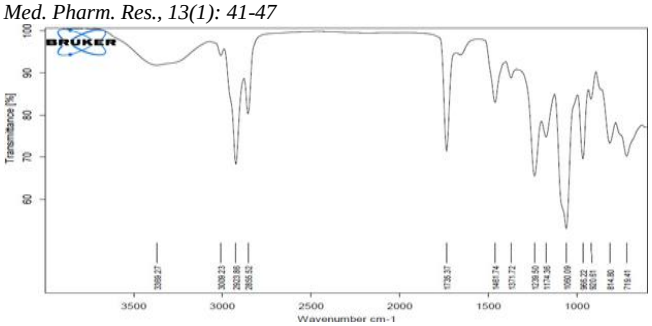

Fig.6 FTIR spectra of physical mixture

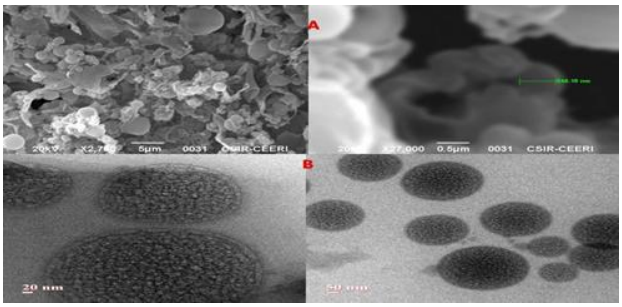

Fig.7 Microscopic morphology of optimized formulation [A] SEM [B] TEM

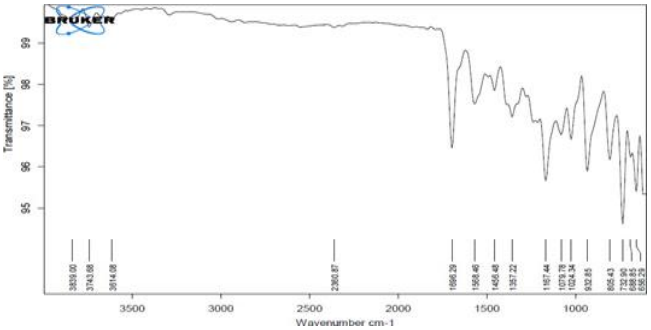

Fig.8 FTIR spectra of Sildenafil

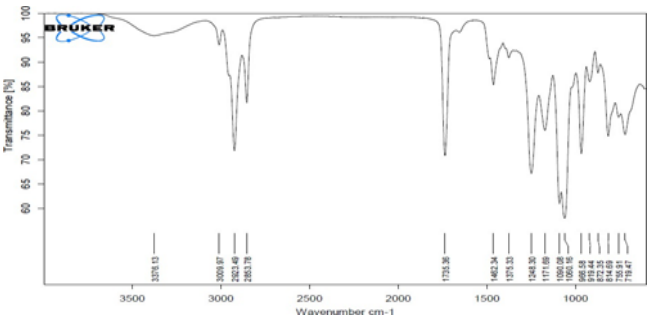

Fig.9 FTIR spectra of Phospholipon 90-G

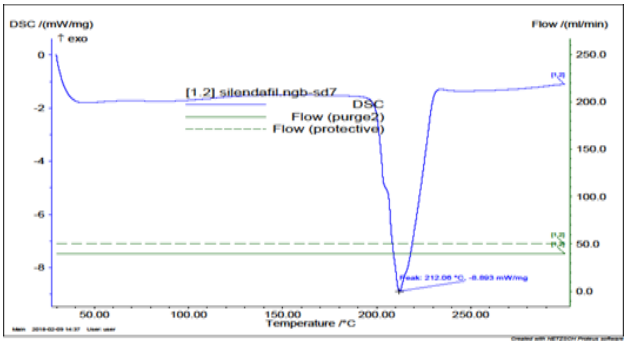

Fig.10 DSC thermogram of Sildenafil

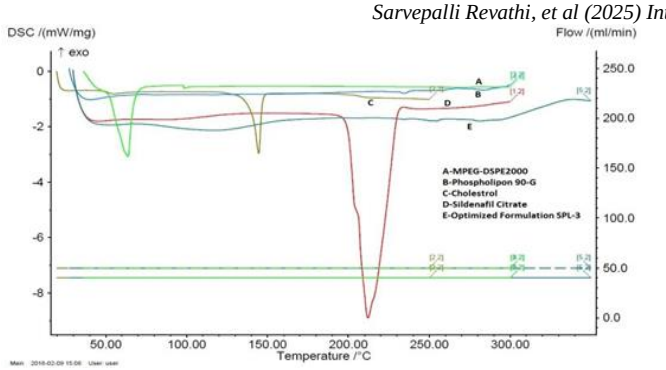

Fig.11DSC thermogram of MPEG-DSPE2000, Phospholipon 90-G, Cholesterol, Sildenafil citrate, & optimized formulation

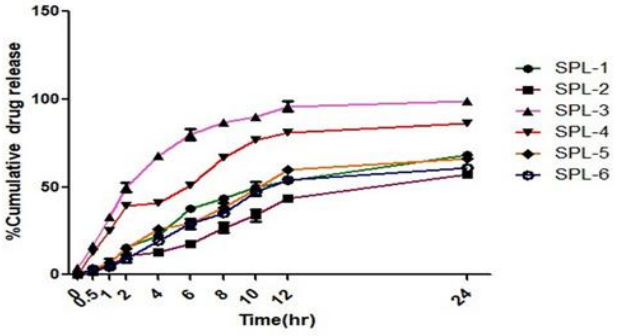

Fig.13 In-vitro drug release of the optimized formulation SPL-3

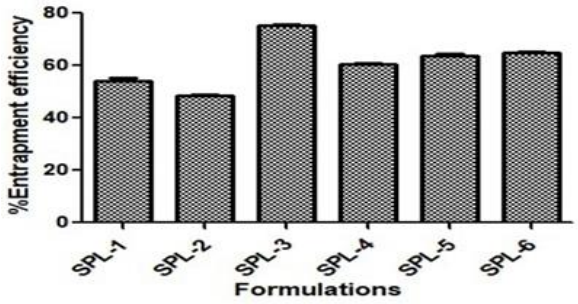

Fig.12 Percentage encapsulation efficiency of Sildenafil loaded PEGylated liposome

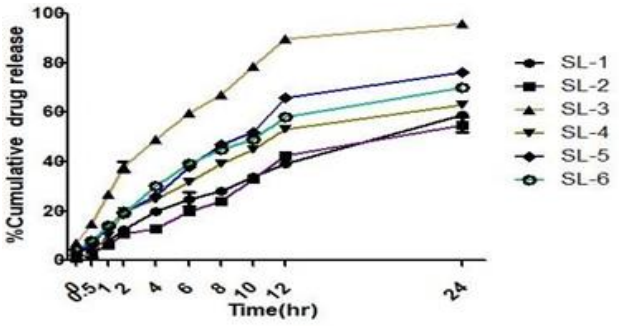

Fig.14 In vitro drug release of the optimized formulation SL-3

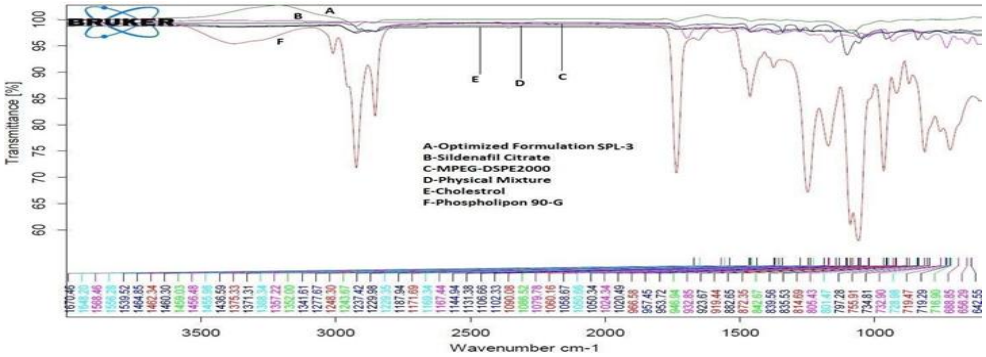

Fig.15: FTIR Spectra of [A]Optimized Formulation[B]Drug[C]MPEG-DSPE 2000[D]Physical Mixture[E]Cholestrol

Table.1 Composition of the PEGylated liposome loaded with Sildenafil

Material	SL-1	SL-2	SL-3	SL-4	SL-5	SL-6
Lipid molar ratio	(9:1)	(9:2)	(9:1:0.25)	(9:2:0.25)	(9:1:0.50)	(9:2:0.50)
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
Leciva DSPC(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

Table.2 Characterization of PEGylated liposome of Sildenafil

Characterization	**SPL-1**	**SPL-2**	**SPL-3**	**SPL-4**	**SPL-5**	**SPL-6**
Vesicle size	162±1.4	168±2.7	155±3.2	177±4.6	181±3.4	211±2.5
PDI	0.256±0.02	0.281±0.04	0.186±0.05	0.232±0.03	0.252±0.05	0.309±0.06
Zeta potential	-19.4±0.8	-21.1±0.5	-32.2±0.3	-26.4±0.2	-24.2±0.8	-21.9±0.6
%EE	57.13±0.4	50.02±1.6	75.89±0.5	60.45±2.4	63.39±0.8	65.74±0.9
%cumulative drug release	68.23±0.02	56.18±1.8	95.09±0.5	87.56±0.9	66.85±1.4	65.74±0.08

Table.3 Composition of PEGylated liposome containing SL/DPPC

Material	SL/DPPC-1	SL/DPPC-2	SL/DPPC-3	SL/DPPC-4	SL/DPPC-5	SL/DPPC-6
Lipid molar ratio	(9:1)	(9:2)	(9:1:0.25)	(9:2:0.25)	(9:1:0.50)	(9:2:0.50)
Drug (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
Leciva DPPC(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

Table.4 Characterization of PEGylated liposomes

Characterization	SL-1	SL-2	SL-3	SL-4	SL-5	SL-6
Vesicle size	116.40±2.8	104.38±3.4	91.14±1.4	109.63±1.6	124.52±2.9	131.09±3.7
PDI	0.486±0.07	0.541±0.04	0.478±0.02	0.524±0.03	0.618±0.06	0.624±0.05
Zeta potential	-24.3±0.4	-28.7±0.3	-30.9±0.7	-19.4±0.4	-17.8±0.2	-16.4±0.6
%EE	49.06±1.5	47.32±0.8	78.65±2.6	53.65±2.4	63.87±1.4	59.51±0.9
% cumulative drug release	59.08±0.16	54.94±3.05	95.96±0.95	63.09±0.75	76.09±0.64	70.09±0.76

4. **Conclusion**

PEGylated liposomes synthesized via the thin film hydration method demonstrated optimal particle size, PDI, zeta potential, and high encapsulation efficiency, making them ideal for enhancing the plasma half-life of sildenafil. The optimized formulation (SL-3) exhibited superior vasorelaxation effects and greater efficacy in reducing mean systolic arterial pressure compared to sildenafil and Revetio®. These PEGylated liposomes offer benefits such as sustained drug release, improved bioavailability, reduced dosage frequency, enhanced stability, and better patient compliance with minimal side effects. Their nanosized nature allows for parenteral administration and prolonged

5. Bibliography

[1] Jackson G, Gillies H, Osterloh I. Past, present, and future: A 7-year update of Viagra® (sildenafil citrate). *Int J Clin Pract*. 2005, 59(6):680–91.

[2] Uthayathas S, Karuppagounder S, Thrash BM, Parameshwaran K, Suppiramaniam V, Dhanasekaran M. Versatile effects of sildenafil: Recent pharmacological applications. *Pharmacol Rep*. 2007, 59(2):150–63.

[3] Kadhim TJ, Khalf OAA. A review search of sildenafil uses in human and in the veterinary medicine. *AIP Conf Proc*. 2023, 2475:100010.

[4] Simonca L, Tulloh R. Sildenafil in infants and children. *Children*. 2017;4(8):60.

[5] Ghofrani HA, Osterloh IH, Grimminger F. Sildenafil: From angina to erectile dysfunction to pulmonary hypertension and beyond. *Nat Rev Drug Discov*. 2006, 5(8): 689–702.

[6] Gong B, Ma M, Xie W, Yang X, Huang Y, Sun T, et al. Direct comparison of tadalafil with sildenafil for the treatment of erectile dysfunction: A systematic review and meta-analysis. *Int Urol Nephrol*. 2017, 49(10):1731–40.

[7] Saikia Q, Hazarika A, Mishra R. A review on the pharmacological importance of PDE5 and its inhibition to manage biomedical conditions. *J Pharmacol Pharmacother*. 2022, 13(4): 246–57.

[8] Delhaye S, Bardoni B. Role of phosphodiesterases in the pathophysiology of neurodevelopmental disorders. Mol Psychiatry. 2021, 26(12): 4570–82.

[9] Nandi S, Kumar P, Amin S, Jha T, Gayen S. First molecular modelling report on tri-substituted pyrazolines as phosphodiesterase 5 (PDE5) inhibitors through classical and machine learning based multi-QSAR analysis. *SAR QSAR Environ Res*. 2021, 32(12): 917–39.

[10] Pasmanter N, Iheanacho F, Hashmi MF. Biochemistry, Cyclic GMP 2023.

[11] Corbin JD. Mechanisms of action of PDE5 inhibition in erectile dysfunction. *Int J Impot Res*. 2004, 16(Suppl 1): S4–7.

[12] Francis SH, Blount MA, Corbin JD. Mammalian cyclic nucleotide phosphodiesterases: Molecular mechanisms and physiological functions. *Physiol Rev*. 2011, 91(2): 651–90.

[13] Ückert S, Kuczyk MA. Cyclic nucleotide metabolism including nitric oxide and phosphodiesterase-related targets in the lower urinary tract. In: *Urinary Tract*. Berlin/Heidelberg: Springer, 2011. p. 527–42.

[14] Nelson MD, Rader F, Tang X, Tavyev J, Nelson SF, Miceli MC, et al. PDE5 inhibition alleviates functional muscle ischemia in boys with Duchenne muscular dystrophy. *Neurology*. 2014, 82(23): 2085–91.

[15] Fernandez-Marcos PJ, Auwerx J. Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis. *Am J Clin Nutr*. 2011;93(4):884S–90S.

[16] Whitaker RM, Wills LP, Stallons LJ, Schnellmann RG. cGMP-selective phosphodiesterase inhibitors stimulate mitochondrial biogenesis and promote recovery from acute kidney injury. *J Pharmacol Exp Ther*. 2013, 347(3): 626–34.

[17] Olmos Y, Sanchez-Gomez FJ, Wild B, Garcia-Quintans N, Cabezudo S, Lamas S, et al. SirT1 regulation of antioxidant genes is dependent on the formation of a FoxO3a/PGC-1α complex. *Antioxid Redox Signal*. 2013;19(13):1507–21.

[18] Kwak YD, Wang R, Li JJ, Zhang YW, Xu H, Liao FF. Differential regulation of BACE1 expression by oxidative and nitrosative signals. *Mol Neurodegener*. 2011, 6: 17.

[19] Nunes AKS, Rapôso C, Rocha SWS, Barbosa KPDS, Luna RLDA, da Cruz-Höfling MA, et al. Involvement of AMPK, IKβα-NFκB and eNOS in the sildenafil anti-inflammatory mechanism in a demyelination model. *Brain Res*. 2015, 627:119–33.

[20] Zhang J, Xie Z, Dong Y, Wang S, Liu C, Zou MH. Identification of nitric oxide as an endogenous activator of the AMP-activated protein kinase in vascular endothelial cells. *J Biol Chem*. 2008, 283(36): 27452–61.

[21] Pușcașu C, Ungurianu A, Șeremet OC, Andrei C, Mihai DP, Negreș S. The influence of sildenafil–metformin combination on hyperalgesia and biochemical markers in diabetic neuropathy in mice. *Medicina*. 2023, 59(7):1375.

[22] Wang L, Chopp M, Zhang ZG. PDE5 inhibitors promote recovery of peripheral neuropathy in diabetic mice. *Neural Regen Res*. 2017;12(2):218–9.

[23] Haider M, Elsherbeny A, Pittalà V, Fallica AN, Alghamdi MA, Greish K. The potential role of sildenafil in cancer management through EPR augmentation. *J Pers Med*. 2021, 11(7):585.

[24] Urla C, Stagno MJ, Fuchs J, Warmann SW, Schmid E. Combination therapy of doxorubicin and sildenafil inhibits the growth of pediatric rhabdomyosarcoma. *J Cancer Res Clin Oncol*. 2022, 149(8): 2513–22.

[25] Das A, Durrant D, Mitchell C, Mayton E, Hoke NN, Salloum FN, et al. Sildenafil increases chemotherapeutic efficacy of doxorubicin in prostate cancer and ameliorates cardiac dysfunction. *Proc Natl Acad Sci U S A*. 2010, 107(42): 18202–7.