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EFFECT OF LIPID COMPOSITION ON LIPOSOMAL FORMULATION OF PRAMIPEXOLE

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ABSTRACT

Treatment of Parkinson's disease (PD) with non ergot dopamine agent, Pramipexole is popular due its advantages over Levodopa therapy. In the present investigations, Pramipexole containing liposomes were formulated to improve the efficacy of the treatment. The effect of varying concentration of cholesterol, soy lecithins and drug on the physical and chemical characteristics of the liposomes was investigated. Liposomal formulations containing varied amounts of Cholesterol, Soy lecithin and Pramipexole were prepared using thin film hydration method. All the formulations were characterized for shape, vesicle Size, TEM, zeta potential, soy lecithin content, percentage entrapment efficiency, in-vitro release studies and drug leakage studies. It was found that the increase in cholesterol concentration increased the vesicle size and percentage entrapment of drug, but the percentage drug release as well leakage of drug were decreased. Increase in soy lecithin concentrations exhibited increase in vesicle size and percentage entrapment of Pramipexole but decreased in vitro release and drug leakage. The concentrations of Pramipexole used however exhibited variable effects on characteristics of liposomes.

KEYWORDS: Pramipexole, Soy Lecithin, Cholesterol, Liposomes.

1. INTRODUCTION

Treatment of neurogenerative diseases like parkinson's disease (PD) has always been a challenge due to blood brain barrier. Almost one in every 100 persons above the age of 65 year is affected by PD which is the second most common neurodegenerative disease after Alzheimer's disease.[1] The signs and symptom of PD are associated to a progressive loss of

dopamine in the basal ganglia. Hence exogenous substitution of dopamine with dopamine agonists or the dopamine's prodrug i.e levodopa, is used to treat the mechanical disorders at the early stage of the PD. Levodopa after administration is converted into dopamine and is stored in dopaminergic neurons.[2] However the prolonged use of levodopa is associated with motor complications. The use of non ergot dopamine agonists Pramipexole delays the onset of these motor complications and is therefore widely used in the PD therapy. Liposomes have achieved significant attention as drug delivery carriers because they are nontoxic, biocompatible, can deliver both hydrophilic and lipophilic drug molecules, protect their cargo from degradation by plasma enzymes, and transport their load across biological membranes and the BBB.[3,4] In the present studies we aim to formulate liposomes of Pramipexole, so as to improve the efficacy of the treatment by enhanced drug concentration in brain and minimal side effects and systemic exposure.

2. MATERIALS AND METHODS

Pramipexole Hydrochloride was obtained as a gift sample from Torrent Research Center, India. Soy lecithin was purchased from TCI chemicals (USA), Cholesterol was purchased from Sigma Aldrich, India. All other chemicals and reagents were of AR grade.

2.1 Preparation of Pramipexole Liposomes

The different ratios of cholesterol, soy lecithins and Pramipexole were used for the preparation of liposome by thin film hydration technique as depicted in Table 1. Multilamellar vesicles were prepared by thin film hydration method using rotary vacuum evaporator.[5] To prepare lipid phase mixture accurately weighed quantities of Pramipexole (1.05 mg – 0.5 M/2.11 mg – 1 M/4.22 mg – 2 M/6.33 mg – 3 M), cholesterol (0.967 mg – 0.25 M/1.935 mg – 0.5 M/3.87 mg – 1 M) and soy lecithin [soy lecithin (1.61 mg – 0.25 M/3.22 mg – 0.5 M/6.44 mg – 1 M); were carefully transferred to the round bottom flask and 10 ml chloroform was added to it. The solvent mixture was removed from lipid phase by evaporation using rotary vacuum evaporator at 50 ± 2°C for 30 min under reduced pressure of 25mm Hg at 100 rpm in order to obtain thin lipid film on the wall of the flask. Subsequently flask was kept under the vacuum over night to ensure complete removal of the residual solvent. The resulting dry film was hydrated with 30 ml phosphate buffer saline pH 7.4 at 25°C. Dispersions thus obtained were vortexed for about 2 min, sonicated for 15 min at room temperature and kept undisturbed at room temperature for almost 4 h to allow complete

swelling of film and to obtain complete vesicular suspension. The liposomes thus prepared were transferred to container and stored in refrigerator.

Table 1: Formulation code and composition of liposomes prepared

Sr. No.	Formulation Code	Composition (ratio by molecular weight)		
		Pramipexole	Soy lecithin	Cholesterol
1	LNS-1	1	1	0.25
2	LNS-2	1	1	0.5
3	LNS-3	1	1	1
4	LNS-4	0.5	1	1
5	LNS-5	2	1	1
6	LNS-6	3	1	1
7	LNS-7	1	0.5	1
8	LNS-8	1	0.25	1

2.2. Evaluation of liposomal formulations

2.2.1. Morphology

Morphology of liposomes was studied under digital optical microscope. All the batches of liposomes were observed under microscope to determine shape. A drop of liposomal dispersion was kept on a glass slide and viewed under the digital microscope (Motic Microscope version 3.2). The results obtained were confirmed by TEM studies.

2.2.2. Vesicle size

Vesicle size of prepared formulation were determined by photon correlation spectrophotometer, which analyses the fluctuations in light scattering due to the brownian motion of the particles using a Zeta sizer ZS 90 (Malvern Instrument Ltd., UK). The formulation was diluted with double distilled water (1:100) and light scattering was monitored at a 90 angle. All measurements were made in triplicate.

2.2.3. Transmission Electron Microscopy (TEM)

The morphological characterization of the liposomes was performed with Transmission Electron Microscopy (TEM) (Jeol Model JM 2100) at SAIF, Kochi. An extremely small amount of liposomes were suspended in water/ethanol to obtain slightly turbid solution. The solution was sonicated to disperse the particles and a drop of the solution was casted on carbon-coated grids of 200 mesh and liposomal morphology was characterized under High Contrast Transmission Electron Microscopy (TEM).

2.2.4. Zeta Potential

Zeta potential was determined filling NE in Folded Capillary Cell using Zetasizer ZS 90, (Malvern Instrument Ltd., UK) by Electrophoretic Light Scattering (ELS) technique. Droplet size of prepared formulation were determined by photon correlation spectrophotometer, which analyses the fluctuations in light scattering due to the brownian motion of the particles using a Zeta sizer ZS 90 (Malvern Instrument Ltd., UK). The formulation was diluted with double distilled water (1:100) and light scattering was monitored at a 90 angle. All measurements were made in triplicate.

2.2.5. Phospholipid content

The phospholipid content of liposomes was determined by UV-Visible Spectrophotometer (Model UV-1700, Shimadzu, Japan) using 1, 2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) as standard according to earlier reported colorimetric method based on a complexation of soy lecithins and ferrothiocyanate.[6] The liposomal suspensions (2 ml) following centrifuge and separation were dried in vacuum oven overnight and the dry residue was dissolved in 2 ml chloroform and 1 ml thiocyanate reagent [27.03 g ferric chloride hexahydrate ($FeCl_3.6H_20$) and 30.4 g ammonium thiocyanate (NH_4SCN) in deionized distilled water diluted to 1 liter]. The contents were then vortex mixed for 1 minute and centrifuged at 2000 rpm for 5 minutes. The lower chloroform layer was separated and absorbance was read at 486 nm. The amount of DPPC was calculated from the regression equation obtained from the calibration curve (10-100 µg/ml) of DPPC using chloroform as blank.

2.2.6. Drug Entrapment

The liposomal suspensions (1 ml) were centrifuged at 2.8 x 10^5 g (Compufuge, Remi, India) for 20 min at 4°C and washed with buffer to obtain Pramipexole containing liposomes free from unentrapped Pramipexole. The supernatant was discarded and fresh pH 7.4 phosphate buffer solution was added. The same procedure was carried out three times to ensure complete removal of unentrapped drug. The loaded concentration of Pramipexole was determined by dissolving Pramipexole containing liposomes in absolute alcohol to obtain clear solution and analyzed by validated HPLC method.

2.2.7. In-Vitro release

In vitro drug release studies were performed using modified Franz diffusion cell (Receptor compartment- 17 ml). Dialysis membrane was placed between the lower cell reservoir and

the glass cell top (Diameter- 1.6 cm, area- 2.0096 cm^2) containing the sample and secured in place with a pinch clamp. The liposomal suspensions (1 ml) were centrifuged at 2.8 x 10^5 g for 20 min at 4°C and washed with buffer to obtain liposomes free from unentrapped drug. Liposomes were then re-dispersed in fresh 1 ml phosphate buffer pH 7.4. Liposomal dispersion (1 ml) was then placed in donor compartment and the receptor compartment was filled with phosphate buffer pH 7.4 (17 ml). The diffusion cell was maintained at 37+0.5°C with stirring at 500 rpm throughout the experiment. Receptor fluid (100 µl) was withdrawn from the receiving compartment at predetermined time points for the period of 5 h and analyzed by validated HPLC method. After each removal, the receptor compartments were replaced with 100 µl fresh phosphate buffer pH 7.4.

Pramipexole in-vitro release kinetics was computed (PCP Disso version 3.1 and DDSolver-Excel based programmes) by using zero order kinetic (Equation 1), first order kinetic (Equation 2), Higuchi square root of time (Equation 3), Hixson-Crowel model (Equation 4) and Korsemeyer-Peppas model (Equation 5).

$Q_t = K_0 . t$ 1

$\ln Q_t = \ln Q_0 - K_1 . t$ 2

$Q_t = K_H . \sqrt{t}$ 3

$\sqrt[3]{Q_0} - \sqrt[3]{Q_t} = K_{HC} . t$ 4

$Mt/M\infty = k.t^n$ 5

Where, Q_t Amount of drug released in time t

Q_0 Initial amount of drug in the system

K_0 Constant for zero order drug release

K_1 Constant for first order drug release

K_H Constant for Higuchi square root time dependant drug release

K_{HC} Constant for Hixson-Crowell drug release model

M_t/M^{∞} Fraction of drug released at time t over the total amount of released drug

Mt Fraction of drug released at time t

$M\infty$ Total amount of drug in the system

k Constant of apparent release

n Diffusion exponent

2.2.8. Drug Leakage

The drug leakage study was carried out at three different temperatures (4°C, 25°C and 37°C). Liposomal dispersion (30 ml) of different batches were made free from unentrapped drug and washed subsequently before reconstituting them in fresh phosphate buffer pH7.4 (30 ml). Unentrapped drug free liposomal dispersion (10 ml) from different batches was transferred to three different amber colored vials each containing 10 ml. The batches were then stored at three different temperature conditions (4°C, 25°C and 37°C) in the incubator and were evaluated daily for drug content for 10 days. Every day 1 ml of liposomal suspension [previously shaken and briefly sonicated (1 min) for dispersal] were withdrawn and centrifuged. The supernatant was removed and separated liposomes were dissolved in methanol (10 ml) and diluted suitably with mobile phase and analyzed by injecting in HPLC column for the estimation of Pramipexole.

2.2.9. Data Analysis

Data were expressed as the mean standard deviation (±SD). The group means were compared and evaluated by the unpaired *t*-test. The statistical significance of differences among more than two groups was determined by one-way ANOVA. A value of $P < 0.05$ was considered to be significant.

3. RESULTS AND DISCUSSION

3.1. Vesicular morphology and size

The TEM images of liposomes prepared with soy lecithin (LNS 1-8) are shown in Figure 1, liposomes were observed as well-identified perfect spheres and exist in disperse as well as aggregated collection. In all the formulations, the presence of spherical shaped vesicles was predominant. Vesicular aggregation was observed with lower cholesterol (LNS-1) content whereas with the increasing cholesterol into the formulations reduced the aggregation process among the vesicular structures (LNS-3). The vesicles prepared with varying Pramipexole content were observed as spherical individual entities without aggregation, whereas liposomes prepared with varying soy lecithin demonstrated lesser vesicular formation with reduced or no aggregation. These results have also been corroborated by other authors.[7,8] and suggests that cholesterol is a stabilizing agent in the lipid bilayer and may reduce the aggregation of liposomes.

3.2. Vesicular size

The average mean diameter for the liposome prepared with varying Pramipexole, soy lecithin and cholesterol by film hydration method was between 180 - 260 nm. Table 2 shows the size distribution of Pramipexole loaded conventional vesicles prepared at 50°C. In formulation LNS-1, 11.11% w/w of cholesterol was added and particle size was found out to be 206.39nm whereas in formulation with 20% w/w cholesterol content (LNS-2), the vesicular diameter was 231.64nm. Further increase in cholesterol content to 33.33% w/w in LNS-3, the vesicular diameter was calculated as 249.27nm. It was observed that all liposomal formulations had similar particle size distribution. The mean hydrodynamic diameters of the liposomes were in the desired particle size range of 180 - 260 nm having monodisperse size distribution with more uniformity observed in the formulation prepared with equal drug, soy lecithin and cholesterol content. This shows that increasing cholesterol results into increased vesicle size. Several theories can be taken into consideration for this result.[9] It has been shown that the addition of steroids to liposomes eliminates the phase transition temperature of soy lecithin and so increases the range of the gel state of vesicles, which therefore remain in a solid sate and prevents the partial dilution of the bilayers.[10] This might have caused increase in vesicle size of liposomes containing more cholesterol. Also the decrease in soy lecithin decreased the vesicle size, this may be due to the decreased amount of phospholipids content into the lipid bilayer.

Table 2: The physical chemical characteristics of liposomal formulations.

S. No.	Vesicular size (diameter nm)±SD	Zeta Potential (mV)	Entrapment Efficiency (%)	Phospholipid content (%)
LNS1	206.39	-41.3 ± 3.85	53.01 ± 1.481	56.83 ± 0.587
LNS2	231.64	-31.7 ± 1.99	55.95 ± 1.414	58.33 ± 0.455
LNS3	249.27	-17.1 ± 5.47	62.18 ± 4.312	71.19 ± 0.589
LNS4	253.66	-17.5 ± 3.36	72.07 ± 4.647	63.51 ± 3.751
LNS5	250.91	-25.4 ± 2.11	24.13 ± 2.282	64.59 ± 0.749
LNS6	256.18	-26.1 ± 6.64	16.07 ± 0.554	64.01 ± 0.415
LNS7	213.54	-16.6 ± 2.23	41.53 ± 3.189	52.43 ± 3.418
LNS8	189.26	-15.4 ± 3.92	39.05 ± 3.421	51.62 ± 2.606

3.3. Transmission Electron Microscopy

Liposomal formulations prepared by thin film hydration and examined by TEM demonstrated well-identified spherical, dispersed as well as aggregated collection (Figure 1). However in most of the formulations, the presence of spherical-shaped vesicles was predominant. Aggregated vesicular structures were observed in the formulations with lower content of

cholesterol. Increase vesicular size was seen in the formulation with increasing Pramipexole content, whereas irregularity in shapes was evident in formulations with lower soy lecithin content.

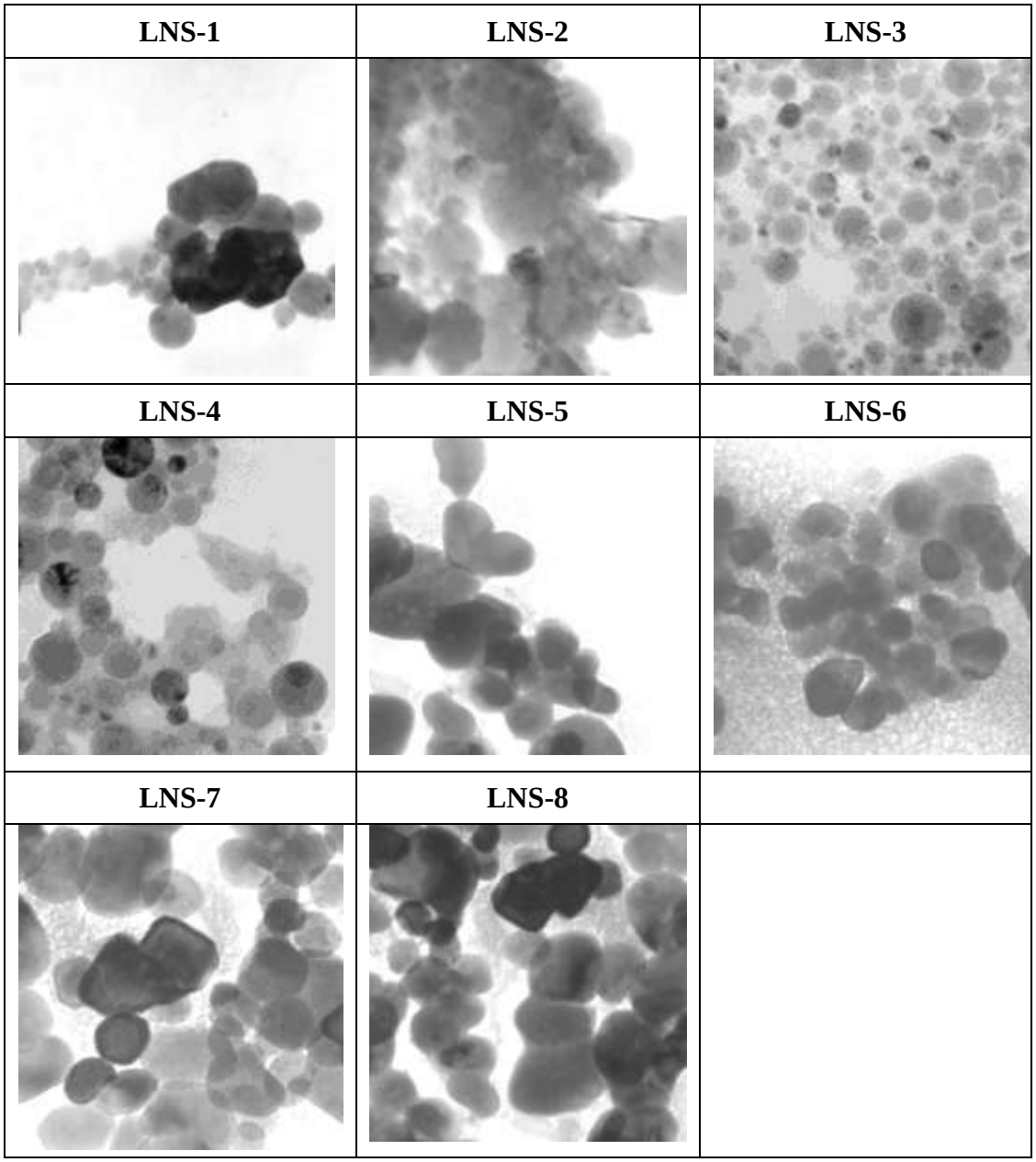

Figure 1: TEM images of the liposomal vesicles prepared with Pramipexole, soy lecithin and cholesterol at three different levels

3.4. Size distribution and zeta potential

It was observed that the vesicle size determined using zeta-sizer had similar size distribution with minor variations. The average diameters of the liposomes were in the size range of 180 –

260 nm with almost monodispersed size distribution. This shows that changing composition had significant effect on particle size distribution of liposomes.

As depicted in Table 2. zeta potential in all the cases was maintained negative side. Increase in the zeta potential values was observed with increase in cholesterol content whereas decreased values were determined with increasing soy lecithin. Although, insignificant and invariable effect of Pramipexole content on zeta potential was observed, it seems that increased Pramipexole content also had inverse correlation with zeta potential values. Pramipexole-loaded liposomes showed a highly negative zeta potential value as reported in the literature.[11] Thus showing drug interaction with bilayer surface since Pramipexole is partially ionized at pH 7.4.

Moreover, zeta potential can be correlated with vesicular size. Increased liposomal size decreases the effective surface area and hence decreases the surface charge (negative). Similar results were corroborated in earlier published reports.[12] Higher surface charge values have been found to promote aggregation property of vesicles. As observed in TEM images (LNS-1 and LSN-2), agglomeration observed could be attributed to higher surface charge.

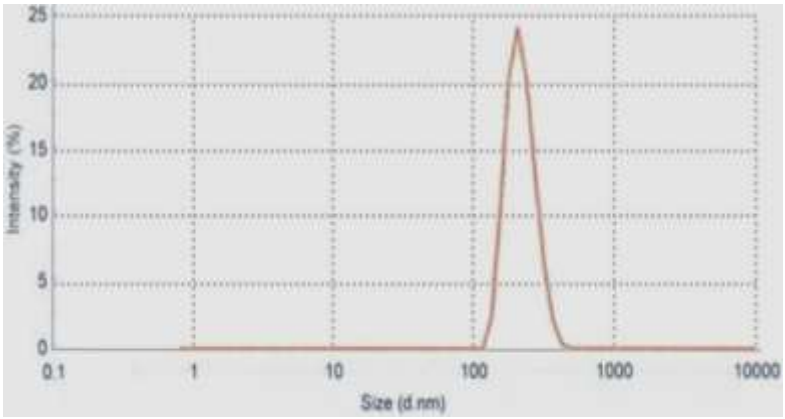

Fig.2 Size distribution and Zeta Potential of LNS-3.

3.5. Phospholipid content

The conventional liposomal vesicles were evaluated for phospholipid content based on complexation between the phospholipid and ferrothiocyanate. As depicted in Table 2, estimated phospholipid content from the vesicles prepared with soy lecithin (LNS-1 to LNS-8) was between 50% and 72%. Increased cholesterol (LNS-1 to LNS-3) increases the phospholipid content in the vesicles complimenting its role of cementing the phospholipid bilayer and therefore increased cholesterol increases the incorporation of phospholipid in the

vesicles.[13] Pramipexole incorporated during film formation demonstrated invariable effect. Whereas highest phospholipid content (71.19 ± 0.589) was estimated in LNS-3, vesicular formulation LNS-4 (63.51 ± 3.751), LNS-5 (64.59 ± 0.749) and LNS-6 (64.01 ± 0.415) demonstrated almost similar phospholipid content. Lower and almost similar phospholipid content was calculated from the formulations with less soy lecithin (LNS-7 and LNS-8) added during film formation.

3.6. Entrapment Efficiency

The drug entrapment of the prepared liposomes was calculated using linearity plot (y = 54.105x - 454.51). Accordingly, the effect of cholesterol, Pramipexole and soy lecithin on entrapment was determined and efficiency was calculated as the fraction of entrapped drug to the total drug added and expressed as percentage (Table-2).

The percent Pramipexole entrapment efficiency was found to be influenced by amount of cholesterol added during film formation. Increase in the cholesterol increased the Pramipexole entrapment (%) in the liposome from 53.01 ± 1.481 to 62.18 ± 4.312 (LNS-1 to LNS-3). This may be due to the fact that increase in cholesterol concentration in the lipid bilayer increases the rigidity of lipid bilayer which causes higher stability and diminished permeability of the liposomal membrane leading to enhanced drug entrapment.[14] Highest entrapment (62.18 ± 4.312%) was calculated from the vesicles prepared with equimolar concentration of Pramipexole, cholesterol and soy lecithin (LNS-3). Further, keeping proportion of cholesterol and soy lecithin constant and varying the Pramipexole content during film formation demonstrated variable entrapment with almost consistent and lower amount of Pramipexole entrapped in vesicles present in 1 ml of liposomal suspension. However, percent fraction entrapped was significantly altered. Highest entrapment efficiency (%) was calculated for LNS-4 (72.07 ± 4.647), whereas further increase in Pramipexole demonstrated decrease in the entrapment efficiency (LNS-5 - 24.13 ± 2.282; LNS-6 - 16.07 ± 0.554). The effect of soy lecithin can be correlated with percent entrapment efficiency as decreased soy lecithin was associated with lowered percent efficiency of Pramipexole entrapment (LNS-3, LNS-7 and LNS-8). This may be attributed to the fact that lower soy lecithin content results into smaller aqueous core volume of liposomes, which can entrap less amount of Pramipexole.[15] The amount of Pramipexole calculated in the vesicles from 1 ml of liposomal suspension LNS-3 (43.733 ± 3.035 µg), LNS-7 (29.203 ± 2.241 µg) and LNS-8 (27.457 ± 2.405 µg) can be correlated with decreased vesicular formation with decreased

amount of soy lecithin. Taken together, these results suggest highest Pramipexole entrapment can be achieved when drug, cholesterol and soy lecithin are used in equimolar proportion.

3.7. In Vitro Drug Release

Composition variability demonstrated significant effect on Pramipexole release from liposomes. In formulation LNS-1, LNS-2 and LNS-3 with 11.11% w/w, 20% w/w and 33.33% w/w cholesterol, the maximum Pramipexole release was 73.18 ± 2.72%, 69.19 ± 0.47% and 58.38 ± 0.83% respectively. As depicted in Figure 3, cholesterol content of liposomal vesicles had significant effect on Pramipexole release and was inversely correlated with it. Doubling the cholesterol ratio (LNS-1, LNS-2 and LNS-3) decreased the release rate. Liposomes containing lower cholesterol exhibited the fastest release among all liposomal dispersions with varying cholesterol. Approximately 15–20% of the drug was released within first 30 min. In agreement with this, higher diffusion flux was observed within first 30min for LNS-1 (122.89 ± 10.65 ng/cm^2/min), LNS-2 (120.15 ± 13.15 ng/cm^2/min) and LNS-3 (110.98 ± 5.79 ng/cm^2/min). Similar drug release profiles from liposomes have been described in the literature demonstrating an initial fast drug release followed by slower release rates of drug.[16,17] Predicted time required for 50% of the drug release (T_{50}) from the release data was 111.77 ± 2.11 min, 156.71 ± 2.25 min and 225.11 ± 5.41 min for LNS-1, LNS-2 and LNS-3 respectively. Extrapolation of release profile for calculating T_{90} (time required for 90% of the drug release) suggested the extension of Pramipexole release with increasing cholesterol content in the liposomal formulations LNS-1 (471.18 ± 36.96 min), LNS-2 (508.28 ± 13.07 min) and LNS-3 (724.14 ± 18.59 min). Since, cholesterol has cementing role in liposomal formulation it improves the integrity of the vesicles, also the presence of cholesterol in the lipid bilayer above phase transition temperature of phospholipid, alters the membrane fluidity by limiting the movement of relatively mobile hydrocarbon chains, reducing membrane permeability and decreasing the efflux of the entrapped drug, resulting in the drug retention. Thus, drug release decreases with increased cholesterol.[14,18] Ordered, compact and integrated vesicular membrane in formulations containing higher amount of cholesterol seems to determine the diffusion flux as well as release of Pramipexole.

Further, the effect of varying drug content was investigated by analyzing the release profiles of LNS-3, LNS-4, LNS-5 and LNS-6. Release data for Pramipexole from liposomal dispersions in phosphate buffer are shown in Figure 3. It was observed that vesicles prepared

with increasing Pramipexole during film formation exhibited higher drug release at 360 min. The maximal average cumulative drug released at 360 min for vesicular formulation LNS-4 (20% w/w Pramipexole), LNS-3 (33% w/w Pramipexole), LNS-5 (50% w/w Pramipexole) and LNS-6 (60% w/w Pramipexole) was 54.52 ± 0.67%, 58.38 ± 0.83%, 64.83 ± 0.41% and 72.45 ± 0.76% respectively. Initial phase of drug release was rapid and burst which can be suggested from the calculated flux of 74.57 ± 7.01 ng/cm^2/min, 110.98 ± 5.79 ng/cm^2/min, 103.78 ± 16.48 ng/cm^2/min and 121.63 ± 6.29 ng/cm^2/min during first 30 min for LNS-4, LNS-3, LNS-5 and LNS-6 respectively. Similarly the computation of T_{50} and T_{90} values for LNS-4, LNS-3, LNS-5, LNS-6 were 270.62 ± 5.32 min and 929.86 ± 62.69 min, 225.11 ± 5.41 min and 724.14 ± 18.59 min, 163.58 ± 2.17 min and 565.89 ± 13.19 min, 132.92 ± 3.17 min and 446.06 ± 16.66 min suggested the increased Pramipexole increases the release rate from vesicles. It is apparent from this data that Pramipexole being hydrophilic agent, initial as well as total cumulative release was the function of entrapped drug which was proportionately increased with increase in the Pramipexole content in the mixture subjected to film formation.

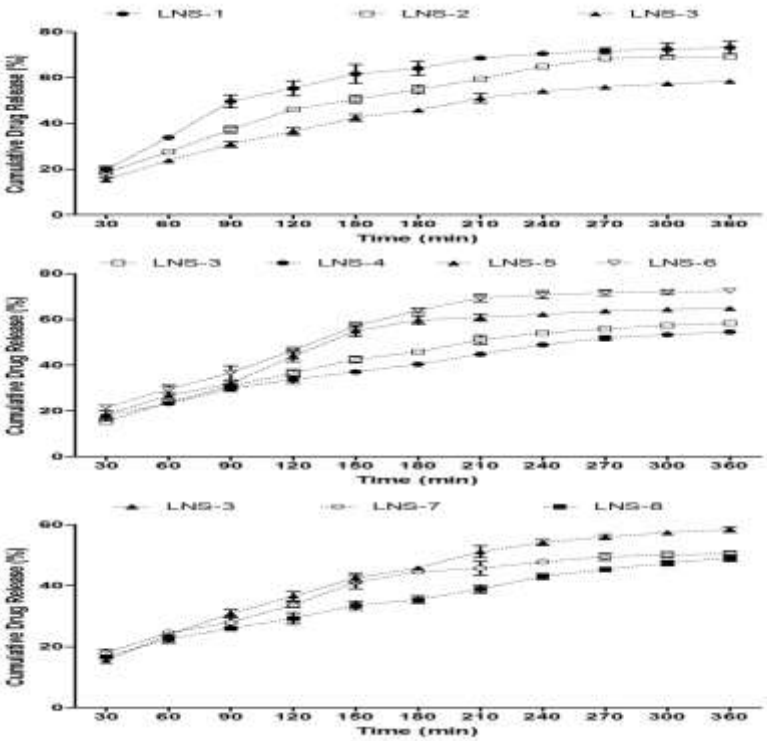

Figure 3 Cumulative in-vitro Pramipexole release from liposomal formulations LNS-1 to LNS-8

In-vitro release data expressed as percent Pramipexole released over 6 h determined for the liposomes containing varying soy lecithin content are shown in Figure.3. The cumulative drug release had significant effect of soy lecithin content. Release of 50.49 ± 0.95%, 49.09 ± 1.09% and 58.38 ± 0.83% of the Pramipexole was calculated from the liposomes containing 20% w/w (LNS-7), 33% w/w (LNS-3) and 50% w/w (LNS-8) respectively. Determination of average flux for the predetermined sampling point demonstrated higher flux of 86.54 ± 6.44 ng/cm^2/min, 110.98 ± 5.79 ng/cm^2/min and 75.89 ± 5.83 ng/cm^2/min for LNS-7, LNS-3 and LNS-8 during initial 30 min. Calculated T50 values for LNS-7, LNS-3 and LNS-8 were 288.26 ± 11.24 min, 225.11 ± 5.41 and 352.99 ± 10.13 min whereas extrapolated T90 values computed was 1170.49 ± 31.22 min, 724.14 ± 18.59 min and 1255 ± 70.88 min respectively which may be due to reduced initial and substantial flux of drug release.

The initial fast rate of release is commonly ascribed to drug detachment from liposomal surface,[16,17] while the later slow release results from sustained drug release from the inner lamellae. Zero-order, first-order, Higuchi, Peppas, Hixon-crowell and Korsemeyer-Peppas equations were applied to in-vitro release data to determine the kinetics and mechanism of Pramipexole release from the liposomes. Correlation coefficient (r) values were high in almost all formulation for Higuchi's model and found to be the best fitting model, suggesting that drug transport out of the liposomes was driven mainly by a diffusion-controlled mechanism. To further validate the domination of the diffusion controlled mechanism of release, the exponent (n) values for Korsemeyer-Peppas kinetic model were calculated and compared. The exponent values for all the formulation were equal to or less than 0.5, suggesting Fickian diffusion mechanism where the drug release was predominantly governed by Pramipexole concentration. The flux data calculated implies the Fickian diffusion mechanism in which initial burst release is due to higher liposomal drug content which decreases gradually over the period of time resulted into substantial decreased flux of drug release subsequently.

The release profiles of liposomal dispersions were biphasic, showing a relatively large burst effect over the first hour, followed by a slower release phase. The burst effect varies with the liposomal composition. Inclusion of cholesterol reduced the release rate, the effect being dependent on the molar ratio of drug, cholesterol and soy lecithin. The release process was characterized by higher correlation coefficients for the Higuchi equation, indicating a Fickian

diffusion controlled release model. Overall the drug entrapment, vesicular morphology and size distribution, drug release suggests the optimal performance for LNS-3.

3.8. Stability Evaluation for Drug Leakage

Liposomes stored at 37°C demonstrated gradually decreased Pramipexole content during the entire period of 10 days whereas lower drug leakage was observed for liposomes stored at 4°C. As shown in Figure 4 cholesterol content controlled the integrity of the liposomes as evident from the lower drug leakage from LNS-3 (33% w/w cholesterol) (7.54 ± 0.11%; 7.63 ± 0.15% and 8.50 ± 0.33%) as compared to LNS-1 (11% w/w cholesterol) (8.75 ± 0.67%, 9.48 ± 0.21% and 11.12 ± 0.73%) and LNS-2 (20% w/w cholesterol) (9.74 ± 0.79%, 9.95 ± 0.72% and 10.49 ± 0.15%) on day 10 for the formulations stored at 4°C, 25°C and 37°C respectively. Moreover, bilayer–drug interaction of the liposomal formulations has been described to influence the stability of the liposomes.[19, 20] Hence we characterized the effect of varying drug incorporation on the stability of liposomes. Formulations prepared with increasing quantities of Pramipexole demonstrated higher drug leakage at all the storage temperatures. Phospholipids form the backbone of the bilayer and hence their stability is important for the stability of liposome. Moreover, aggregation or flocculation also affect the stability of liposomes and may result in loss of encapsulated drug.[21]

In LNS-7 with soy lecithin content was 11% w/w, the percent drug leakage was determined as 6.58 ± 0.64%, 7.06 ± 0.97% and 10.29 ± 0.78% at 4°C, 25°C and 37°C respectively. Pramipexole leakage however was not found to be influenced by the soy lecithin in the quantities used in the formulation LSN-8 (20% w/w soy lecithin) (7.18 ± 0.57%, 6.86 ± 1.05% and 10.92 ± 0.49% at 4°C, 25°C and 37°C respectively) and LSN-3 (33% w/w soy lecithin) (7.54 ± 0.11%; 7.63 ± 0.15% and 8.50 ± 0.33% at 4°C, 25°C and 37°C respectively). In addition to encapsulation efficiency and minimal drug leakage during storage is also an important concern for liposomal formulations.[22] At elevated leakage of drug from the liposomes may be due to the effect of temperature on the gel to liquid transition of lipid bilayer together with possible degradation of phospholipid causing defects in membrane integrity.[23, 24] Permeability and stability of liposomes are influenced by the rigidity/stiffness of the lipid bilayer and hence lipid composition plays significant role in stability. Temperature affects phosphatidylcholine (PC) liposomes stability and incorporation of cholesterol to the liposomes reduces the temperature effects and therefore prevents drug loss as well as liposome aggregation.

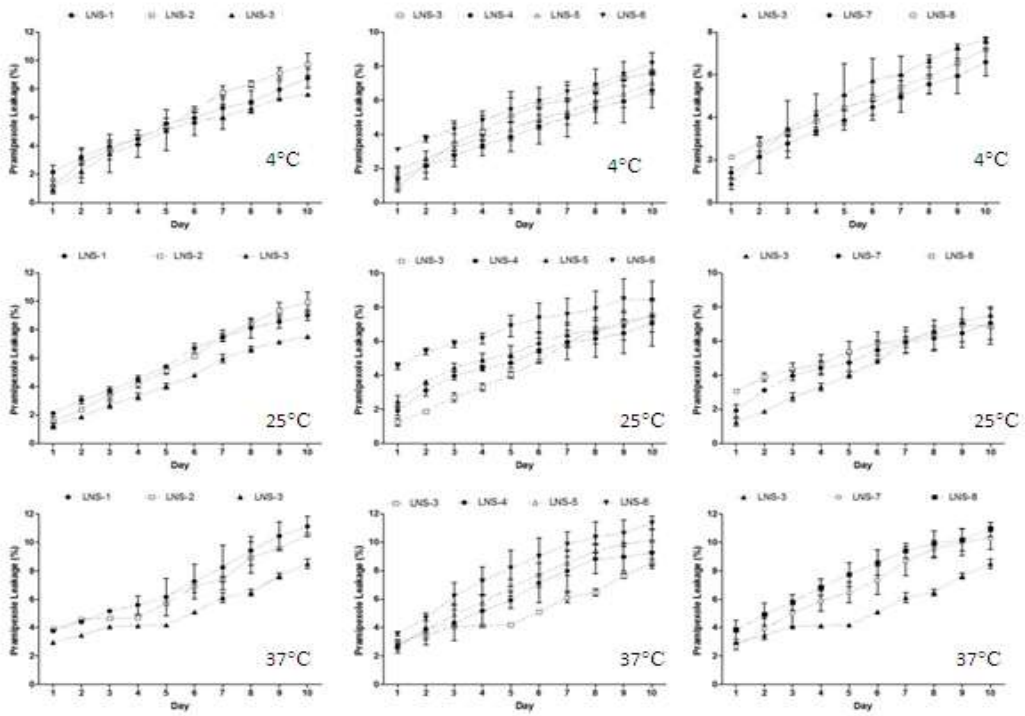

Figure 4 Pramipexole leakage (% ± SD) from the liposomal formulation prepared with soy lecithin stored at 4°C, 25°C & 37°C.

4. CONCLUSION

Conventional Liposomes of Pramipexole were successfully prepared by thin film hydration method. Increase in cholesterol content markedly increased vesicle size, zeta potential, percentage entrapment efficiency and phospholipid content, while drug release and drug leakage showed inverse relation with cholesterol concentration. Similarly decrease in soy lecithin concentration exhibited decrease in diameter, entrapment efficiency as well as phospholipid content of the liposomes, however the effect on zeta potential was not significant. Drug release and leakage decreased with decrease in soy lecithin. Varying the concentration of Pramipexole showed variable effects, hence could not be correlated. The formulation LNS3 exhibited optimum characteristics and release profiles thus could be the promising vehicles for the delivery of Pramipexole.

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