MS.ID-IJPBS-6941
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2024) 14 (3): 86-95
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

Design, Development and *In Vitro* Evaluation of Ropinirole HCl Loaded Liposomes by Central Composite Design

Vasavi Pachika* and Vijaya Ratna Jayanthi

St. Mary's College of Pharmacy, Secunderabad, Telangana, India
Professor, Andhra University, Visakhapatnam, Andhra Pradesh, INDIA.

Received: 15 Mar 2024 / Accepted: 5 Apr 2024 / Published online: 1 Jul 2024
***Corresponding Author Email:** vasavisrinivas14@gmail.com

Abstract

Ropinirole HCl is a non-ergoline dopamine agonist used in Parkinson's disease and restless legs syndrome, has poor bioavailability when administered orally. Liposomes mediated intranasal delivery is considered a potential route of administration to increase the bioavailability of ropinirole HCl. This study aims to optimize ropinirole HCl loaded liposomes using central composite design and were prepared by ethanol injection method. This process leads to an optimum formulation with desired EE, particle size and 100 % invitro drug release. Counter plots and response surface curves display visual diagrammatic relationship between experimental and input variables. Optimized formulation showed EE of 92.1%, particle size 84.6 nm and invitro drug release 94.4%. This study demonstrates that statistical experimental design methodology can optimize the formulation and process variables to achieve favourable response for ropinirole HCl loaded liposomes.

Keywords

Liposomes, Ropinirole HCl, Intranasal, Entrapment Efficacy, Particle size, Central composite designs.

*****

INTRODUCTION:

Research on novel drug delivery systems is continuously evolving with an objective to deliver the drugs at right site on right time. Among other drug deliveries, drug delivery to the central nervous system (CNS) remains a challenge in the treatment of CNS disorders. This challenge is arising because of the formidable mechanisms that are present in the brain, the blood brain barrier (BBB) to protect the brain from entry of foreign substances. The BBB consists of the endothelium of the brain vessels, the basal membrane and neuroglial cells, which restrict the entry of materials into the brain. Majority of the drugs are restricted from reaching the brain because of its impermeability. The BBB is considered to be the major obstacle in the delivery of CNS drugs.[1] CNS diseases like Parkinson's, Alzheimer's, Huntington's, schizophrenia, migraine, brain tumour and meningitis need transport of drugs to the brain for treatment.[2,3]

The intranasal route exploits the unique neural connection of the olfactory and the trigeminal nerves between the nose and the CSF, to deliver drugs to the brain. This route can be exploited as a potential alternative drug delivery route for efficient delivery of challenging drugs, such as, low molecular weight polar compounds, peptides, proteins and large proteins and polysaccharides like vaccines or DNA plasmids. Several scientists around the globe, along with Illum Lisbeth, reported evidences of nose-to-brain transport.[4] Drug absorption across the olfactory region of the nasal mucosa is a good option to preferentially target drugs to the brain.[5-7]

Nasal drug delivery is an attractive approach for the systemic delivery of drugs which suffer from low oral bioavailability because of their extensive hepatic

first-pass metabolism. Intranasal delivery of drugs has been widely studied and a number of reports are available in the literature. Drugs like carbamazepine, dopamine, neurotoxic, metals, local anaesthetics, carboxylic acids and nerve growth factors have been reported to reach the central nervous system, after nasal administration, in experimental animals.[8-11]

The olfactory region of the nasal passages has exclusive anatomic and physiologic features and offers both extracellular and intracellular pathways into the CNS by bypassing the blood-brain barrier.[12]

Despite improved drug absorption from nasal route and its capacity to target brain, there are several limitations like ciliary clearance, enzymatic degradation and low permeability, which restrict the success of this route for drug delivery.

The liposomes are considered as suitable colloidal carrier systems in reducing the mucociliary clearance in the nose and in providing sustained release properties.[13,14]

The relationship between the lipid solubility of a drug and its CNS diffusion is considered to be a direct correlation. Thus, designing a carrier system with lipid vesicles for CNS penetration becomes a good strategy.[15]

Ropinirole hydrochloride (RH) is a non-ergot D2/D3 DA agonist with the greatest affinity at the D3 receptors and is used in patients with moderate or advanced Parkinson's Disease (PD). It is absorbed rapidly with peak plasma concentrations occurring in 1–2 hours. Oral ropinirole therapy is associated with nausea, vomiting, and gastrointestinal disturbances and ropinirole possess low oral bioavailability (approx. 50%) because of the hepatic first pass effect; its adverse effects and frequent dosing schedule contribute to non-compliance among patients.

MATERIALS AND METHODS:

Ropinirole Hydrochloride was provided by Hetero Labs, Hydrogenated soya phosphatidylcholine (HSPC), Di stearoyl phospho Glycerol (DSPG), Cholesterol, all other reagents and chemicals used were of laboratory/analytical or HPLC grade.

Preparation of Ropinirole HCl loaded Liposomes:

Accurately weighed amounts of (HSPC/Lipoid-S-100), DSPG and cholesterol were taken in a 10 mL beaker and dissolved in sufficient quantity of ethanol with slight heating (50-55°C) on a hot plate. Simultaneously, ropinirole hydrochloride was dissolved in 10 mL of SNF pH 6.8 by Magnetic Stirrer (REMI) which was maintained at (1000 to 1200 rpm) at room temperature using a Teflon coated bead. Monophasic ethanolic lipid mixture was injected into the drug solution kept under stirring at the rate of 0.25 mL/min using the 14-gauge needle. The aqueous phase immediately turned milky indicating the vesicle formation. The system was kept under stirring for up to 1.5-2 hours, to facilitate the removal of ethanol. The vesicular dispersion was made up to 10 mL with SNF pH 6.8 and the dispersion was filtered through sterile graded filter (0.22µm EDF filter, Pall Corporation, Pall India Pvt. Ltd., Mumbai, India) to obtain sterilized vesicles. The vesicles were then transferred to 10 CC vials and the transfer was then followed by nitrogen sparging and sealing. The prepared liposomes (batch numbers from HF1 to HF15 were HSPC based ropinirole hydrochloride liposomes whereas LF1 to LF15 were Lipoid-S-100 based ropinirole hydrochloride liposomes) were stored at 4°C, until analysis.[16-18]

Experimental design :

The liposomal formulation was optimized by statistical experimental design (Design Expert® DX10 software) version. Central composite design was utilised for the development of ropinirole hydrochloride loaded liposomes by ethanol injection method. Specified amount of lipid (HSPC/Lipoid-S-100), Cholesterol and DSPG were taken as independent factors (X_1, X_2 and X_3). All the calculations were done at milligrams (mg) level. Amount of drug (50mg) and dispersion medium (10 mL) were kept constant. Vesicle size (Y_1) and Percent entrapment efficiency (Y_2) and cumulative percent *in vitro* drug release (Y_3) were selected as dependent variables. Composition is given in Table 1.

Table 1: Central composite design of ropinirole HCl liposomes

Batch code	Amount of HSPC (mg) X_1	Amount of cholesterol (mg) X_2	Amount of DSPG (mg) X_3
F1	187.5	-0.340	50
F2	187.5	37.5	50
F3	187.5	75.34	50
F4	187.5	37.5	-0.453
F5	187.5	37.5	100.45
F6	75	60	80
F7	75	15	80

F8	75	15	20
F9	75	60	20
F10	300	15	80
F11	300	60	80
F12	300	60	20
F13	300	15	20
F14	376.70	37.5	50
F15	-1.706	37.5	50

Characterization of vesicles

The prepared liposomes were characterized by drug content, vesicle size, zeta potential, percent EE and *in vitro* drug release studies. All the determinations were performed in triplicate and values are represented as average values with standard deviations.

Determination of drug content

A weighed amount of the prepared liposomes, equivalent to 5 mg of ropinirole hydrochloride, was taken in a test tube and was lysed with 9 mL of methanol. Subsequent dilutions (10 mL) were made with the SNF pH 6.8 and the absorbance was measured at a λ_{max} of 250 nm using a UV-VIS spectrophotometer[18] (Shimadzu double beam UV-Spectrophotometer, model-1800, Japan, with UV Probe software). The study was performed in triplicate.

Vesicle size & Zeta Potential:

Mean vesicle size and polydispersity index (PDI) of ropinirole HCl loaded liposomes was determined by dynamic light scattering method using Malvern Nano ZS (Malvern Instruments Ltd., UK). Analysis was carried out at 25°C temperature keeping the angle of detection at 90°. The mean vesicle size is expressed in terms of nm (average of the vesicle size). The size distribution of vesicles was expressed in terms of poly dispersity index (PdI). The zeta potential expressed in terms of surface charge of the system (mV).

Entrapment efficiency

The unentrapped drug was separated from vesicles using ultra centrifugation technique. Vesicular dispersion of volume 5ml was taken and centrifuged at 10000 rpm for 90 min at a controlled temperature of 4°C (Remi cooling centrifuge). Supernatant containing un entrapped drug was withdrawn and measured spectrophotometrically at 250 nm against pH 7.4 phosphate buffer as blank. All the determinations were made in triplicate. The amount of drug entrapped in liposomes was determined by the Eq.

$$\mathbf{EE\ (\%) = [(Cd - C)/Cd] * 100}$$

Where, C_d is the concentration of total drug and C is the concentration of un entrapped drug.

***In vitro* release studies**

The In vitro drug release studies were performed using vertical Franz diffusion cells with an effective diffusional area of 4.52 cm^2 and 28 ml of receptor compartment. 5 mg equivalent dose of ropinirole HCl liposomes was placed in the donor compartment. 25 mL of phosphate buffer pH 6.8 was used as receptor medium to ensure sink condition. The receptor compartment was maintained at 37°C±0.5ºC and stirred by a magnetic stirrer at 100 rpm. The donor compartment was separated from the receptor compartment by cellulose dialyzing membrane (Membra–Cel MD cut-off 10 KD) which was soaked in the receptor medium overnight. At predetermined time intervals (0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hrs). 1 mL aliquots were withdrawn from the sampling port and were replaced with an equal volume of fresh buffer to maintain constant volume. The samples were analysed spectrophotometrically at 250 nm.

Drug release kinetics

In order to understand the kinetics and mechanism of drug release, the data of *in vitro* drug release study of optimized batch of vesicles was fitted in various kinetic equations like Zero order (cumulative % released vs. time), First order (log % drug remaining vs. time), Higuchi's model (cumulative % drug released vs. square root of time) and Peppas (log % drug released vs. log time). K and r values were calculated for the best fit line obtained by regression analysis of the above plots.

FTIR studies

The FTIR (ATIR) (Bruker) technique was used for the study of functional group interaction of drug and excipients. Appropriate amounts of HSPC, cholesterol, DSPG, ropinirole HCl and ropinirole HCl liposomal formulation were studied for FTIR to elucidate the functional group positions between the drug and other used excipients.

Differential scanning calorimetry

DSC is a Thermo analytical technique which provides accurate thermodynamic data and phase transformations. DSC study was carried out for pure ropinirole hydrochloride, individual selected lipids, physical mixture (1:1) and ropinirole hydrochloride liposomal formulation. Appropriate quantity of samples (pure drug, individual lipids, physical mixture of drug with lipids and liposomal formulations) were taken and sealed in a standard pan with a lid. The DSC parameters were evaluated using (Mettler Toledo STAR eSW 8.10, Model DSC 822e) at a heating rate of 10°C/min from 25°C to 250°C. An empty aluminium pan served as reference. Nitrogen was used as a purge gas, at a flow rate of 20 mL/min for all the studies[20].

RESULTS & DISCUSSION:

Vesicle size, PDI and Zeta potential:

The average size of vesicles and the breadth of the particle distribution are crucial parameters for characterization, as they affect both physical stability and permeability across the blood-brain barrier (BBB). The liposome vesicle sizes varied from 84.6±0.9 nm to 381.1±0.2 nm. The polydispersity index (PDI) ranged from 0.102±0.6 to 0.315±0.4, suggesting a homogeneous dispersion. It was noted that the inclusion of negatively charged DSPG had an impact on the vesicle size[21]

The results for average vesicle size and PDI for formulations are showed in Table 2.

Various factors influencing the vesicle size are explained by equation

Vesicle size (Y1) = +584.52-1.49*X1-11.66*X2-5.00*X3-0.01*X1X2-2.11*X1X3+ 0.045*X2X3+5.15*X12+0.160*X22+0.035*X32

The regression coefficient (R2) for the above equation was 0.9129 indicated good correlations between the variables and selected response and model was found to be significant.

The zeta potential values of ropinirole loaded liposomal dispersions were found to be in the range of -14.3±0.8 to -42.2±0.6 mV. For any liquid dosage form surface charge is essential for its stability. The F2 batch liposomes exhibited a higher zeta potential value of -42 mV due to the surface charge imparting nature of the DSPG3. The values of zeta potential showed that vesicles had sufficient charge to inhibit aggregation of vesicles due to electric repulsion. The results of zeta potential are showed in Table 2.

Entrapment efficiency:

The results of drug entrapment efficiency of RHcl loaded liposome formulations are shown in table 2.

All formulation batches with different ratios of HSPG and Cholesterol DSPG were created to assess their impact on the percentage of drug entrapment. The liposomal %EE varied from 38.1±0.2 to 92.1±0.4.

The results regarding the entrapment efficiency of liposomes loaded with ropinirole HCl indicate that the ratio of lipid, cholesterol, and DSPG is essential for achieving a higher encapsulation efficiency. Optimal concentrations of HSPC, cholesterol, and DSPG lead to an increased percentage of encapsulation, as evidenced by the reduced particle size, which may enhance the surface area available for encapsulating ropinirole HCl within the aqueous core of the liposomal vesicles. The polynomial equation for entrapment efficiency is

% EE (Y2) = -8.39+0.30*X1+1.80*X2+1.129*X3+4.04*X1X2-1.48*X1X3-1.00*X2X3- 1.05*X12-0.02*X22-7.3*X32

The regression coefficient of 0.9856 in the equation suggests that the quadratic model is significant, indicating that the ratios of HSPC, cholesterol, and DSPG have a notable impact on entrapment efficiency.

***In-Vitro* drug release studies:**

The cumulative release of R. HCl from loaded liposomes was investigated using the dialysis sac method. The release profile for R. HCl liposomes is presented in Table 2. The drug release testing is a fundamental part of drug product development and manufacture. It is also employed as a quality control tool to monitor batch-to-batch consistency of the drug release from the vesicular systems. The *in vitro* drug release was characterized by Franz diffusion cell which has 4.52 cm^2 of donor compartment and 28 ml capacity of receptor compartment. The *in vitro* drug release profiles of all the ropinirole HCl liposomes are shown in **Fig.1A, 1B & 1C.** The percentage drug release of ropinirole HCl loaded liposomes was found that in the range of 48% to 94.38% respectively.

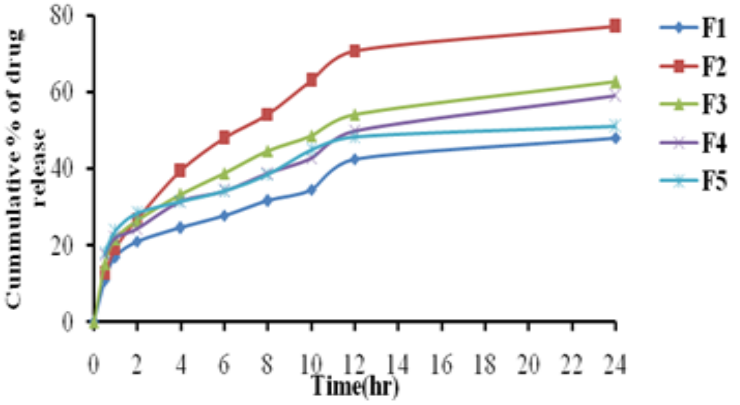

Fig.1A: *In vitro* drug release of F1 to F5

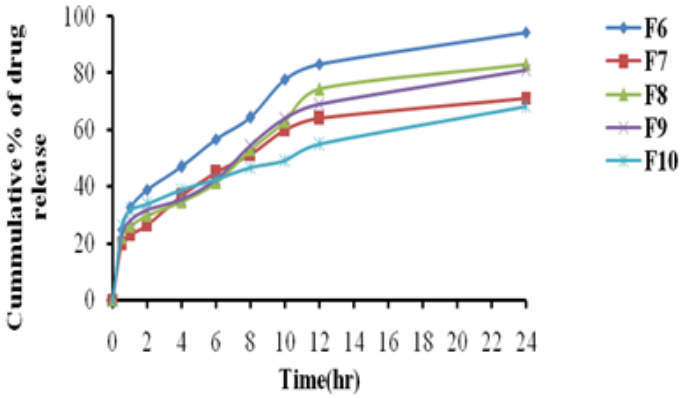

Fig.1B: *In vitro* drug release of F6 to F10

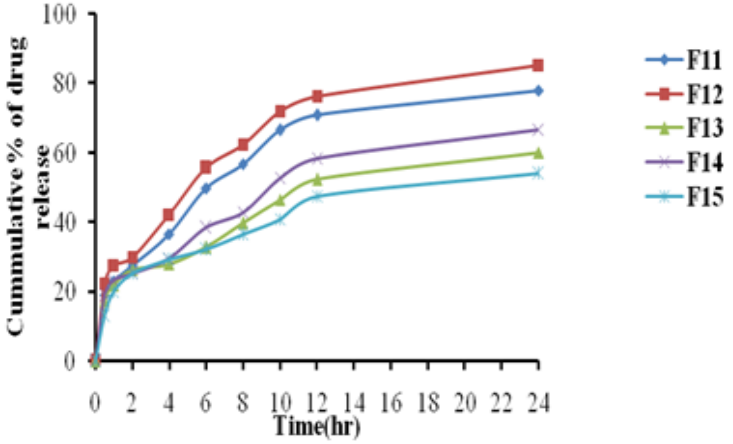

Fig.1C: *Invitro* drug release of F11 to F 15

The mechanism of vesicular drug release is affected by several physicochemical factors, including lipid composition, lamellarity, dispersion medium, and preparation method. This suggests that the lipid composition of liposomes determines their membrane fluidity, which subsequently impacts the rate of drug release. The polynomial equation for percent drug release is

% Drug Release (Y3) =+13.14+0.27*X1+1.54*X2+0.80*X3+3.45*X1X2-1.93*X1X3-7.19*X2X3-9.35*X12-0.022*X22-4.84*X32

The regression coefficient of the equation presented is 0.9714, suggesting that the quadratic model demonstrates a significant and strong correlation between the variables and drug release. Additionally, the chosen model variables were determined to be significant.

Table 2: Composition of liposomes and responses in central composite design

Batch code	X_1	X_2	X_3	Drug Content (%)	Size (nm)	PDI	%EE	Zeta Potential (mV)	100% *in vitro* drug release
F1	75	60	80	98.1±0.5	381.1±0.2	0.112±0.2	38.1±0.2	-16.4±0.8	48.2±0.1
F2	75	15	80	98.9±0.9	238.2±0.6	0.131±0.8	54±0.8	-21.6±1.1	62.57±0.4
F3	75	15	20	99.3±0.1	186.1 ±0.8	0.104±0.6	68.2±0.2	-26.7±0.6	76.98±1.1
F4	75	60	20	97.8±0.3	251.2±0.1	0.214±0.1	55.1±0.2	-19.1±0.4	57.13±1.2
F5	187.5	-0.340	50	99.8±0.8	351.4±0.5	0.186±0.5	45.2±0.6	-14.3 ±0.8	51.12±1.5
F6	187.5	37.5	50	101.2±0.6	84.6±0.9	0.102±0.6	92.1±0.4	-42.2±0.6	94.38±1.4
F7	187.5	75.34	50	96.4±0.7	283.1±1.1	0.194±0.3	68.4±0.8	-24.1±0.1	71.15±1.1
F8	187.5	37.5	-0.453	97.3±0.8	224.3±0.2	0.156±0.4	71.2±0.5	-18.1±0.5	82.8±0.9
F9	187.5	37.5	100.45	98.9±1.0	134.4±0.8	0.161±0.8	76.6±0.4	-34.1±0.6	81.16±0.5
F10	300	15	80	96.3±0.5	262.2±0.3	0.345±0.4	56.4±0.8	-27.8±0.4	68.12±0.3
F11	300	60	80	96.8±0.9	262.3±0.4	0.313±0.6	71.1±0.6	-24.1±0.3	78.10±0.8
F12	300	60	20	99.5±0.1	221.4±0.6	0.288±0.3	86.2±0.2	-21.8±0.4	85.63±1.1
F13	300	15	20	97.6±0.3	282.3±0.6	0.303±0.2	48.3±0.3	-17.8±0.6	60.19±1.4
F14	376.70	37.5	50	99.1±0.8	206±0.2	0.226±0.4	68.4±0.4	-26.4±0.3	66.71±1.3
F15	-1.706	37.5	50	98±0.6	338±0.4	0.223±0.2	41.6±0.1	-19.4±0.2	54.12±1.4

Optimization and validation:

The counter plots and response surface plots clarify how variables influence the responses and their interactions in **Fig.No 2 and 3.** Optimization of the formulation was achieved through the application of desirability and overlay plots. To reach this goal, a multi-criteria decision-making approach was employed, incorporating both a numerical optimization method utilizing the desirability function and a graphical optimization method through the overlay plot **Fig No.4**. Specific minimum and maximum levels were established for each response parameter, and the objectives were integrated into a comprehensive desirability function. The solutions were organized based on their desirability, with the highest rated at 1.0. An analysis of variance (ANOVA) was conducted to determine the significant impact of various factors on the response regression coefficients. To assess the selected experimental design, the optimized formulations were created and evaluated against specific parameters. The experimental outcomes were then compared to the predicted values. The established criteria included maximizing drug entrapment in the formulation, minimizing liposome size (in nanometers), and achieving the highest in vitro drug release after 24 hours. There was no significant statistical difference between the predicted and observed responses, with a relative error of less than 0.5%. The experimental findings aligned well with the predicted values, thereby confirming the model's predictability and validity. From above mentioned evaluation data F6 was considered as optimized batches, since they had minimum size, maximum % EE and maximum in vitro drug release at the end of 24 hrs.

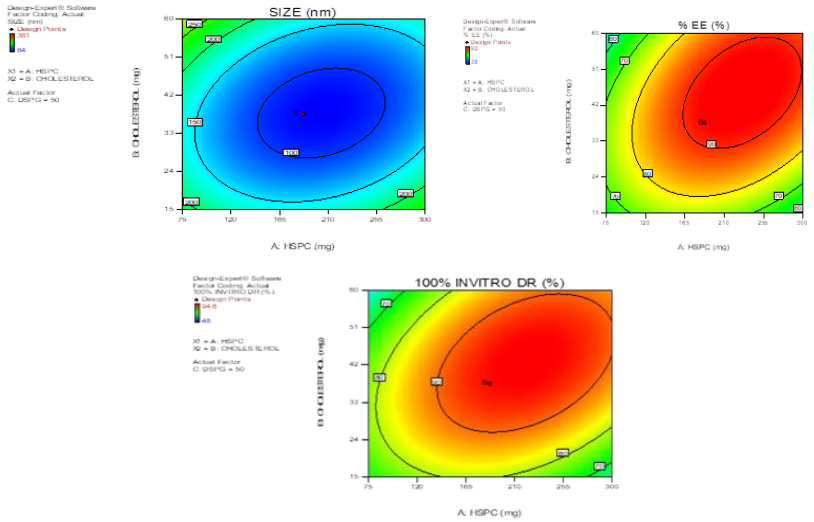

Fig. No 2: Contour plots for the amount of lipid (HSPC) (X_1), amount of cholesterol (X_2) and amount of DSPG (X_3) in liposomal formulations a) on particle size (Y_1), b) on %EE (Y_2) c) on 100% *in vitro* drug release (Y_3)

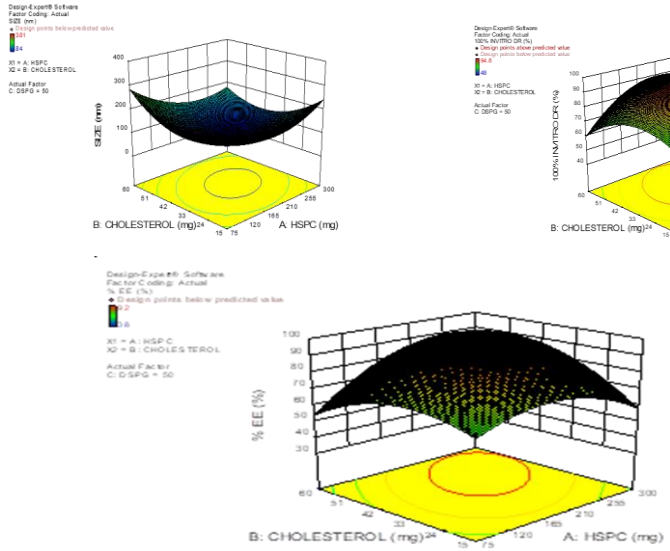

Fig. No 3: Response surface graphs for the amount of lipid (HSPC) (X1), amount of cholesterol (X2) and amount of DSPG (X3) in liposomal formulations a) on particle size (Y1), b) on %EE (Y2) c) on 100% in vitro drug release (Y3)

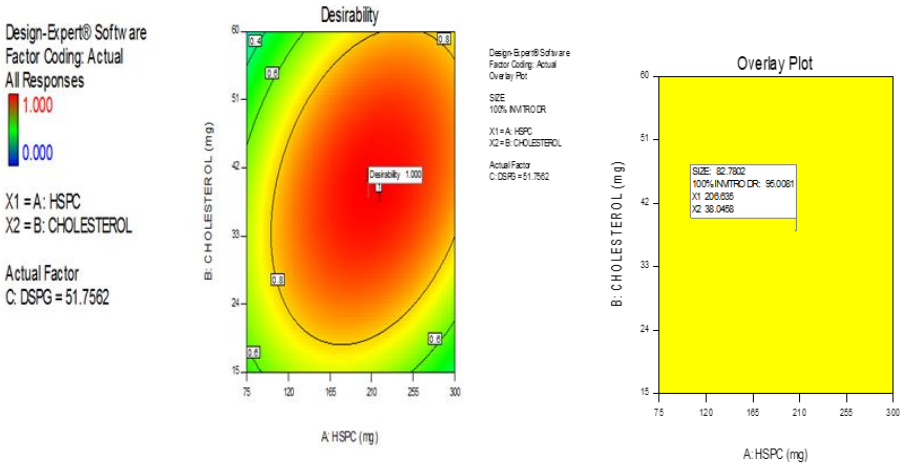

Fig. No: 4 Desirability and Overlay plots of ropinirole HCl liposomes

Drug release kinetics:

Release data were analyzed using kinetic models to explore the kinetics of drug release. The findings indicated that the in vitro drug release was most accurately described by zero-order kinetics, with a correlation coefficient (r) of 0.990. Additionally, the Higuchi model provided the best fit for the drug release, suggesting that the release from vesicles is governed by diffusion. When the release data were evaluated through Korsmeyer-Peppas equation, the release exponent (n) was determined to be 0.600, indicating that the drug release mechanism from the liposomes follows a non-Fickian diffusion process.[22]

SEM:

The shape and morphology of R.Hcl-loaded liposomes (F6) were analyzed using optical microscopy and scanning electron microscopy (SEM). The findings indicated that the vesicles produced were smooth, spherical, and unilamellar.

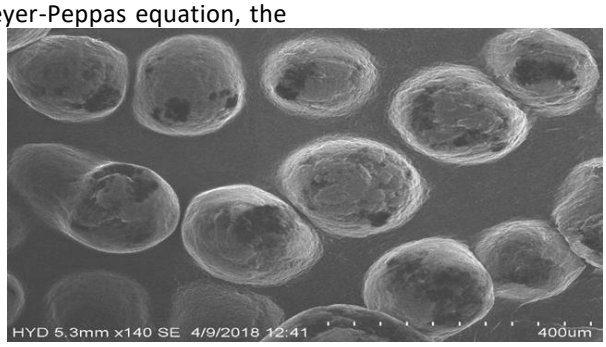

Fig No 5: SEM of Ropinirole Hcl formulation

FTIR:

The FTIR spectrum of the pure drug showed the characteristic FTIR peaks at 3416cm-1 (N-H stretching), 1626cm-1(C=C stretching),3076cm-1(aromatic, C-H stretching), 2938cm-1 and 2881cm-1(aliphatic C-H stretching),1312cm-1 and 1347cm-1 (C-N stretching),1759cm-1(C=O stretching. FTIR results revealed that (**Figure 4.17 to Figure 4.23**), there were no drug excipient interactions, as the above-mentioned peaks at specific wave numbers, were also observed in physical mixtures and formulations developed, using both the lipids, HSPC and DSPG[23]**.**

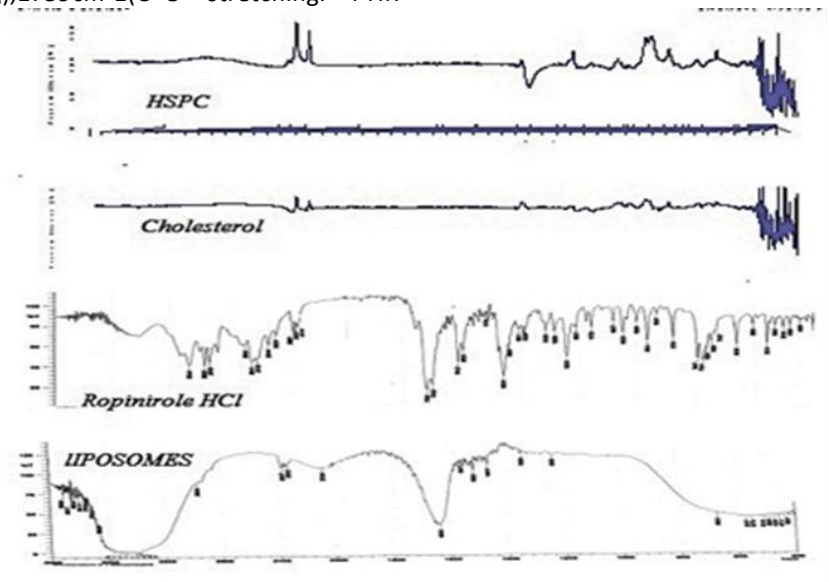

Fig. No: 6 FTIR image of HSPC, Cholesterol, ropinirole HCl and HF6

DSC

Thermal profiles were analysed for plain ropinirole hydrochloride (RHCl), drug-lipid physical mixtures, and ropinirole hydrochloride-loaded liposomes. The differential scanning calorimetry (DSC) thermograms of the pure drug revealed a distinct endothermic peak at 260°C (Curve A), signifying its melting point. The physical mixture of formulation F6, which is based on HSPC, displayed two endothermic peaks at approximately 84.3°C and 155°C. This suggests that ropinirole hydrochloride was partially integrated into the mixture of HSPC, DSPG, and cholesterol. These findings imply a uniform distribution of the drug within the liposomal bilayers, potentially indicating a transformation of the drug into an amorphous state. The DSC thermogram for HF6, which consists of ropinirole hydrochloride-loaded HSPC-based liposomes, exhibited two endothermic peaks at

around 61°C and 85°C, both of which are lower than those observed in the corresponding physical mixture. This further supports the conclusion that the drug was evenly distributed throughout the liposomal bilayers and may have transitioned to an amorphous form.[24,25]

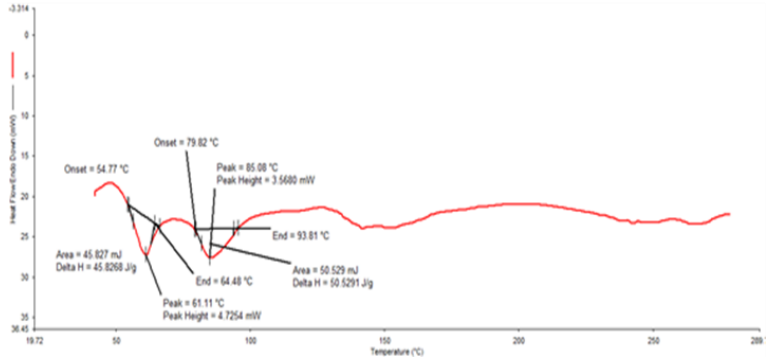

Fig No: 7 DSC Thermogram of Ropinirole HCl Liposome formulation

CONCLUSION:

The ropinirole HCl loaded liposomal system developed, characterised and optimized for various parameters like particle size, entrapment efficacy and % drug release. It can be concluded that. Design-Expert software assisted in designing the experimental protocol with minimal errors. The liposomes were discrete spherical vesicles with smooth surface, unilamellar and size ranging from 84 to 380 nm. Liposomal loaded ropinirole Hcl could be viewed as a robust, safe and stable alternative to conventional dosage forms. Further pharmacokinetic studies are essential to prove the concept.

REFERENCES:

1. Pardridge WM. Blood–brain barrier drug targeting: the future of brain drug development. Mol Interv. 2003; 3: 90–105.
2. Frey WH II. Intranasal delivery: Bypassing the blood–brain barrier to deliver therapeutic agents to the brain and spinal cord. Drug Deliv Technol. 2002; 2: 46–49.
3. Misra A, Ganesh S, Shahiwala A, Shah SP. Drug delivery to the central nervous system: a review. J Pharm Pharm Sci. 2003; 6 (2): 252-73.
4. Illum, L. Nasal drug delivery-possibilities, problems and solutions. J Control Rel. 2003; 87(1-3): 187-198.
5. Van den Berg, Merkus P. Romeijn S.G, Verhoef J.C, Merkus F.W. Hydroxocobalamin Uptake into the Cerebrospinal Fluid after Nasal and Intravenous Delivery in Rats and Humans. J Drug Target. 2003; 11 (6): 325-31.
6. Dahlin M., Bjork, E. Nasal administration of a physostigmine analogue (NXX-066) for Alzheimer's disease to rats. Int J Pharm. 2001; 212 (2): 267-274.
7. Bagger M.A., Bechgaard, E. The potential of nasal application for delivery to the central brain-a microdialysis study of fluorescein in rats. Eur J Pharm Sci. 2004; 21 (2-3): 235-42.
8. Chien YM and Chang Y. Historical Developments of Transnasal Systemic Medications, in Trans nasal Systemic Medications: Fundamentals, Developmental Concepts and Biomedical Assessments (Ed. Y. W.Chien), Elsevier, Amsterdam.1985; 1–100.
9. Barakat NS, Omar SA and Ahmed AAE. Carbamazepine uptake into rat brain following intra-olfactory transport. J Pharm Pharmacol. 2005; 58: 63–72.
10. Dahlin M, Bergman U, Jansson B, Bjork E and Brittebo E. Transfer of dopamine in the olfactory pathway following nasal administration in mice. Pharm Res. 2000; 17: 737–742.
11. Henriksson J and Tjalve H. Uptake of inorganic mercury in the olfactory bulbs via olfactory pathways in rats. Environ Res. 1998; 77: 130–140.
12. Chou KJ and Donovan MD. Lidocaine distribution into the CNS following nasal and arterial delivery: a comparison of local sampling and micro dialysis techniques. Int J Pharm. 1998; 171: 53–61.
13. Thorne RG, Emory CR, Ala TA and Frey WH. Quantitative analysis of the olfactory pathway for drug delivery to the brain. Brain Res. 1995; 692: 278–282.
14. Gregoriadis G, Florence A.T. Liposomes in drug delivery. Clinical, diagnostic and ophthalmic potential. Drugs.1993; 45:15–28.
15. Schnyder, A, Huwyler, J. Drug transport to brain with targeted liposomes. NeuroRX. 2005; 2: 99–107.
16. Shadab A. Pathan, Zeenat Iqbal, Syed M. A. Zaidi, Sushma Talegaonkar, Divya Vohra, Gaurav K. Jain, Adnan Azeem, Nitin Jain, Jigar R. Lalani, Roop K. Khar and Farhan J. Ahmad. CNS Drug Delivery Systems: Novel Approaches Recent Patents on Drug Delivery; Formulation. 2009; 3: 71-89.
17. Miquel Pons, Merce Foradada and Joan Estelrich. Liposomes obtained by the ethanol injection method. Int J Pharm. 1993; 95: 51-56.
18. Catherine Charcosset, Audrey Juban, Jean-Pierre Valour,Sébastien Urbaniak, Hatem Fessi. Preparation of liposomes at large scale using the ethanol injection method: Effect of scale-up and injection devices. Chem Eng. Sci. 2015; 94: 508- 515.

19. Shmuel Batzri and Edward D.Korn. Single bilayers liposomes prepared without sonication. Biochimica Biophysica Acta. 1973; 298: 1015-1019.
20. Shaikh KS, Atmaram P. Liposomal delivery enhances cutaneous availability of ciclopiroxolamine. Lat Am J Pharm. 2010; 29 (5): 763-770.
21. Dole MN, Patel PA, Sawant SD, Shedpure PS. Advance applications of fourier transform infrared spectroscopy. Int J Pharm Sci Rev Res, 2011; 7 (2): 159-166.
22. Chen J, Ping QN, Guo JX, Chu XZ, Song MM, Effect of phospholipid composition on characterization of liposomes containing 9-nitrocamptothecin. Drug Dev Ind Pharm. 2006. 32(6): 719-26.
23. Anderson M, Omri A. The effect of different lipid components on the in vitro stability and release kinetics of liposome formulations. Drug Deliv. 2004; 11(1): 33–39.
24. M. Mohan Varma and M. Santosh Kumar. Formulation and Evaluation of Matrix Tablets of Ropinirole Hydrochloride for Oral Controlled Release. IJPP. 2015; 2(1): 27-42.
25. Chandrakant sing V. Pardeshi, Veena S. Belgamwar, Avinash R. Tekade, Sanjay J. Surana. Novel surface modified polymer–lipid hybrid nanoparticles as intranasal carriers for ropinirole hydrochloride: in vitro, ex vivo and in vivo pharmacodynamic evaluation. J Mater Sci Mater Med. 2103; 24 (9): 20101-15.
26. Jain SA, Chauk DS, Tekade AR, Gattani SG. Formulation and evaluation of nasal mucoadhesive microspheres of Samaritan sauccinate. J. Microencapsul.2009;26:711-21.