RESEARCH ARTICLE

Repurposing Ropinirole HCl for Targeting Breast Cancer: Preparation and Characterization of Liposomal Formulation Based on QBD Approach

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ABSTRACT

Breast cancer remains a leading cause of cancer-related mortality worldwide, necessitating the development of novel therapeutic strategies. Ropinirole HCl, a dopamine receptor agonist primarily used for Parkinson's disease, has shown potential anticancer activity by modulating dopamine receptors, inhibiting angiogenesis, and inducing apoptosis in breast cancer cells. This study aims to formulate and optimize a liposomal delivery system for Ropinirole HCl using the Quality by Design (QbD) approach. A Box-Behnken design was employed to evaluate the effects of lipid concentration, stabilizer concentration, and homogenization speed on critical quality attributes, including particle size, zeta potential, and polydispersity index (PDI). The ideal combination (F12) was 75.8 nm in size, -38.6 mV in surface charge, and 0.559 in PDI, meaning that it is more stable and more suitable as a targeted drug carrier. These findings indicate the fact that liposomal Ropinirole HCl can become an excellent method of breast cancer treatment.

Keywords: Ropinirole HCl, Breast Cancer, Preparation, Characterization, Liposomal, QbD Approach

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INTRODUCTION

Breast cancer is the most common diagnosed type of malignancy among the women in the world representing more than 10 percent of new cancer diagnoses every year. Although the field of cancer treatment has made great achievements in early detection/ molecular profiling/therapeutics approaches, conventional chemotherapy still remains a subject of major challenges, such as systemic toxicity, emergence of multi-drug resistance, and a lack of tumor specificity. Such drawbacks precondition the need to discuss other therapeutic options that would enhance the effectiveness of treatment and reduce the side effects thereof [1,2].

Dopamine receptors have been found to be crucial in tumorigenesis through novel research findings that show that they have a role in regulating the main oncogenic activities like tumor growth, angiogenesis, metastasis, and immune evasion. Out of the several dopamine receptor agonists, Ropinirole Hydrochloride (HCl) has received interest because it selectively activates both dopamine 2/D3 receptors. Ropinirole HCl has been initially studied as a treatment of neurological conditions like Parkinson disease and restless leg syndrome but showed great potential in cancer treatment by preventing the growth of tumor cells and causing apoptosis in dopamine receptor-positive cancerous cells. Nonetheless, poor aqueous solubility, limited bioavailability, and high systemic clearance are some of the cause of additional constraints inherent in the drug that limit its value in clinical applications in oncology[3,4].

To streamline the challenges, the drug delivery systems through nanocarriers, especially liposomes is a possible approach in maximizing the effectiveness and stability of Ropinirole HCl. Some of the advantages offered by liposomal drug delivery systems are enhanced solubility, long term systemic circulation, selective targeting at tumor tissues using enhanced permeability and retention (EPR) effect. Also, liposomal encapsulation ensures that there is slow and controlled drug release and this enhances its therapeutic effect and also ensures that there is reduction of its systemic toxicity. Liposomes have the capability to deliver and protect hydrophobic drugs against enzymatic deactivation besides delivering the encapsulated drugs to the target cells and serve as promising in cancer therapy[5-7].

To guarantee a logical and well-graduated development process, Quality by Design (QbD) approach was used at formulating and optimizing liposomal Ropinirole HCl. QbD is a scientific and systematic approach which simplifies the determination of critical process and formulation parameters that ensure reproducible high quality product. The main formulation variables investigated in this study have been the concentration of lipids and stabilizers and the homogenation speed by systematically altering them in a Box-Behnken design of the experiments to see how they affect the most important quality characteristics which are particle size, zeta potential and polydispersity index. The optimised liposomal formulation was also determined in terms of its physicochemical properties, their stability, and their prospective use in targeted breast cancer medicine. The

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proposed study will contribute to a new method of treatment of breast cancer due to the combination of nanotechnology-based drug delivery with medication on dopamine receptors, which will slightly change the current approach to treating this disease and, hopefully, positively influence patient survival chances [8-11].

Reagents employed: Ropinirole HCl (Active pharmaceutical ingredient, Sigma-Aldrich) was taken as the model drug. The investigated lipid was phosphatidylcholine (Lipoid GmbH), whereas cholesterol (Sigma-Aldrich) was used as the stabilizer. The organic solvents of lipid dissolution were chloroform and methanol (Merck). As a hydration medium, phosphate-buffered saline (PBS, Thermo Fisher Scientific) was employed, whereas all experiments used high-purity distilled water (Millipore). Rotary evaporator (Buchi, Switzerland) was used in formulating the lipid film, a high-speed homogenizer (IKA, Germany) in formulating particle size reduction, a particle size analyzer (Malvern Zetasizer), a zeta potential analyzer (Malvern Instruments), a differential scanning calorimeter (DSC, PerkinElmer) and UV-visible spectrophotometer (Shimadzu, Japan) was used in quantitative determination of Ropinirole HCl.

Table 1: Box-Behnken design for the formulation development and Optimization of a Liposomes of Ropinirole HCl

File Version	13.0.5.0		
Study Type	Response Surface	Subtype	Randomized
Design Type	Box-Behnken	Runs	17.00
Design Model	Quadratic	Blocks	No Blocks
Build Time (ms)	3.00		

Table 2: UV Analysis of Ropinirole HCL

Concentration (µg/mL)	Absorbance at 287nm λ max
0	
2	0.124
4	0.236
6	0.348
8	0.442
10	0.526

MATERIALS AMD METHODS

Preparation of Liposomal Formulation

Lipid and Stabilizer Dissolution

lipids/Phosphatidylcholine and cholesterol were carefully measured to the required concentrations and dissolved in a ratio of 2: 1 of mixture of chloroform and methanol. This would make sure that the lipid components are uniformly dispersed in the organic solvent and a stable structure of lipid bilayers could be achieved.

Film Formation

The lipid solution was placed in a round-bottom flask and evaporated to dryness, at reduced pressure (40o C) using a Buchi rotary evaporator. This step gets the organic solvent removed leading to the separation of the thin lipid film on the inner surface of the flask. The lipid film has to be evenly and without cracks in order to form homogenous liposomal vesicles.

Hydration

Equivalent concentrations of Ropinirole HCl was added to Phosphate-buffered Saline (PBS) that had the dried lipid film hydrated at room temperatures. The flask was kept in constant motion during 1 hour in order to completely hydrate and obtain a milky suspension formed by multilamellar vesicles (MLVs). This is essential in drug entrapment and even distribution.

Homogenization

It used high-speed homogenization by the method of variable speed (500, 1000 and 1500RPM) with an IKA high-speed homogenizer on the liposomal suspension. Homogenization transforms multilamellar vesicle into unilamellar ones that are smaller in size and more homogeneous, increasing the efficiency of entrapment and drug stability. The time of homogenization to avoid the shear stress too high and which may break the liposomes was also optimized[8-10].

Optimization Using Box-Behnken Design

Experimental Design

A Box-Behnken Design (BBD) was used to specifically measure the influence of three experimental factors on changing the liposomal formulation. The independent variables were lipid concentration (10 percent, 20 percent, 30 percent), the stabilizer concentration (5 percent, 10 percent, 15 percent) of the homogenization speed (500 RPM, 1000 RPM, 1500 RPM). The choice of these

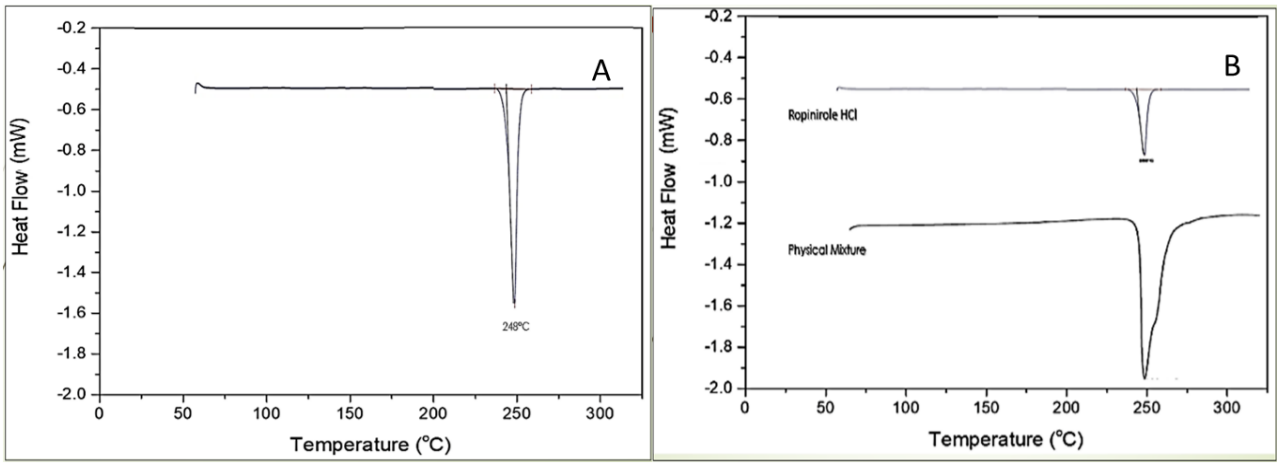

Figure 1: (A) DSC Analysis Ropinirole HCL - 248°C; (B) Physical Mixture - 249°C

parameters was related to the influence on some of the most vital properties of the formulation.

Responses Evaluated:

Particle Size

It utilizes a Malvern Zetasizer in order to obtain nano-sized liposomes that penetrate the tumor effectively.

Zeta Potential

It is determined under a zeta potential analyzer in order to evaluate colloidal stability.

Polydispersity Index (PDI)

Analyzed to give the magnitude of uniformity of particle size.

Experimental Runs

There were 17 run experimental runs and three center points which were carried out to analyze the interplays of independent variables. The design structured a process of evaluating the impact of each of the parameters systematically and it was able to provide an optimal formulation that possessed necessary physicochemical properties.

Data Analysis

It was determined the significance of the independent variables together with their interactions through statistical analysis (Analysis of variance (ANOVA)) process. The formulation parameters were optimized through the application of regression modeling that determined predictive equations[11-14].

UV Analysis of Ropinirole HCl

Quantitative study of Ropinirole HCl was done by applying the UV-Visible Spectrophotometer (Shimadzu UV-1800) at the wavelength of 287 nm. Ropinirole HCl (100 5g/mL) stock solution was prepared in phosphate buffer (5G.C) of pH 7.4 and serial dilutions performed to achieve concentrations between 5 and 50 5g/mL. Each solution absorbance was read at 287 nm which was using a 1 cm path length quartz cuvette. To determine the linearity, a calibration curve was plotted in the form of concentration versus absorbance and the value of R 2 was greater than 0.99 which means that it is strongly correlated. This procedure made precise quantification of Ropinirole HCl in the liposomal formulation[15].

Drug and Excipient Compatibility Studies - DSC Analysis

Ropinirole HCl drug-excipient compatibility was tested with the Liposomal excipients utilizing a Differential Scanning Calorimeter (DSC-60, Shimadzu). To prepare the samples, pure Ropinirole HCl, phospholipids and cholesterol, and physical mixtures between the three were weighed (approximately 5 mg) and put in aluminum pans, which were subsequently crimp-sealed to avoid the loss of moisture. During thermal analysis, the samples were heated from 25°C to 300°C at a controlled heating rate of 10°C/min under a nitrogen flow of 50 mL/min. The resulting thermograms were examined for shifts, disappearance, or broadening of endothermic or exothermic peaks, which could indicate potential drug-excipient interactions. The presence of significant alterations in peak patterns suggested incompatibility, whereas the retention of the drug's characteristic thermal behavior confirmed its compatibility with the liposomal formulation[16].

Characterization of Liposomes

Particle Size and Polydispersity Index (PDI)

Particle size and the degree of polydispersity in the liposomal dispersion was also determined using the HORIBA Nanoparticle Size Analyzer SZ-100 by Dynamic

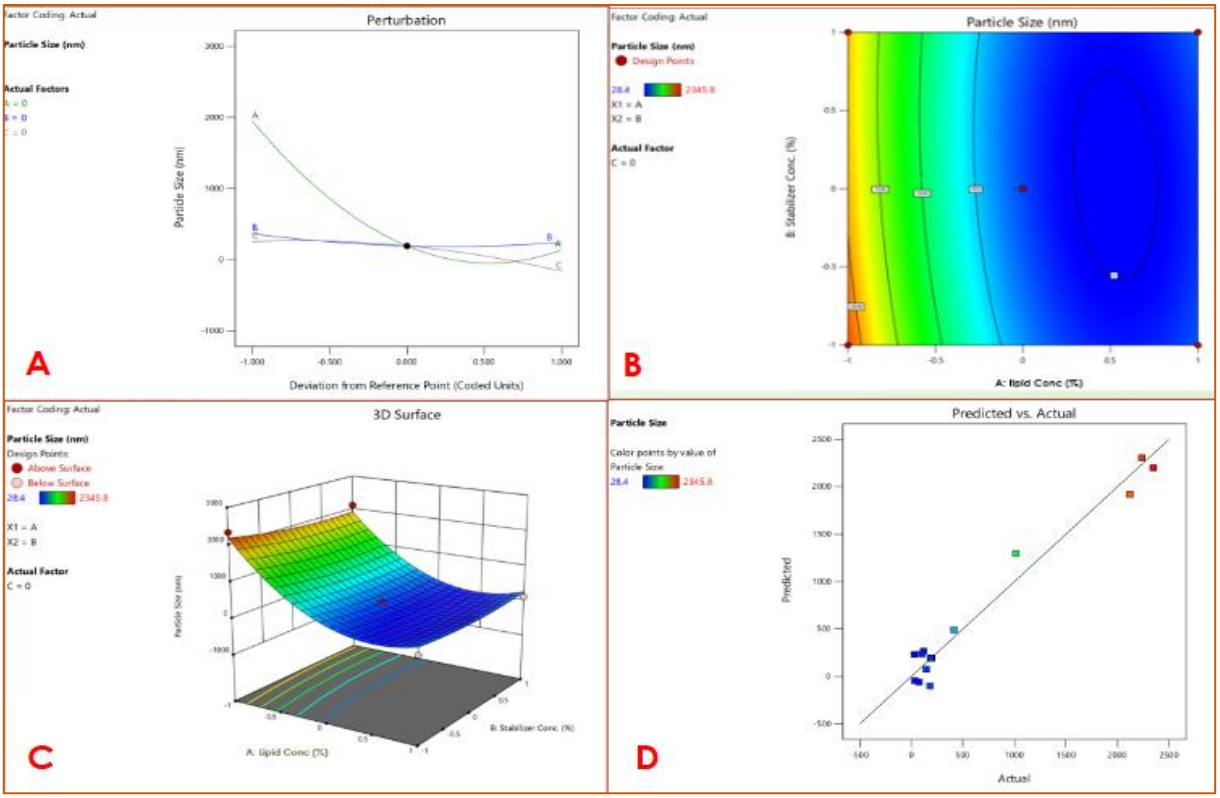

Figure 2: Effect of Variables on Response 1: PS A - Perturbation Plot of; B - Contour Plot; C - 3D Surface Plot; D - Predicted Vs Actual Value

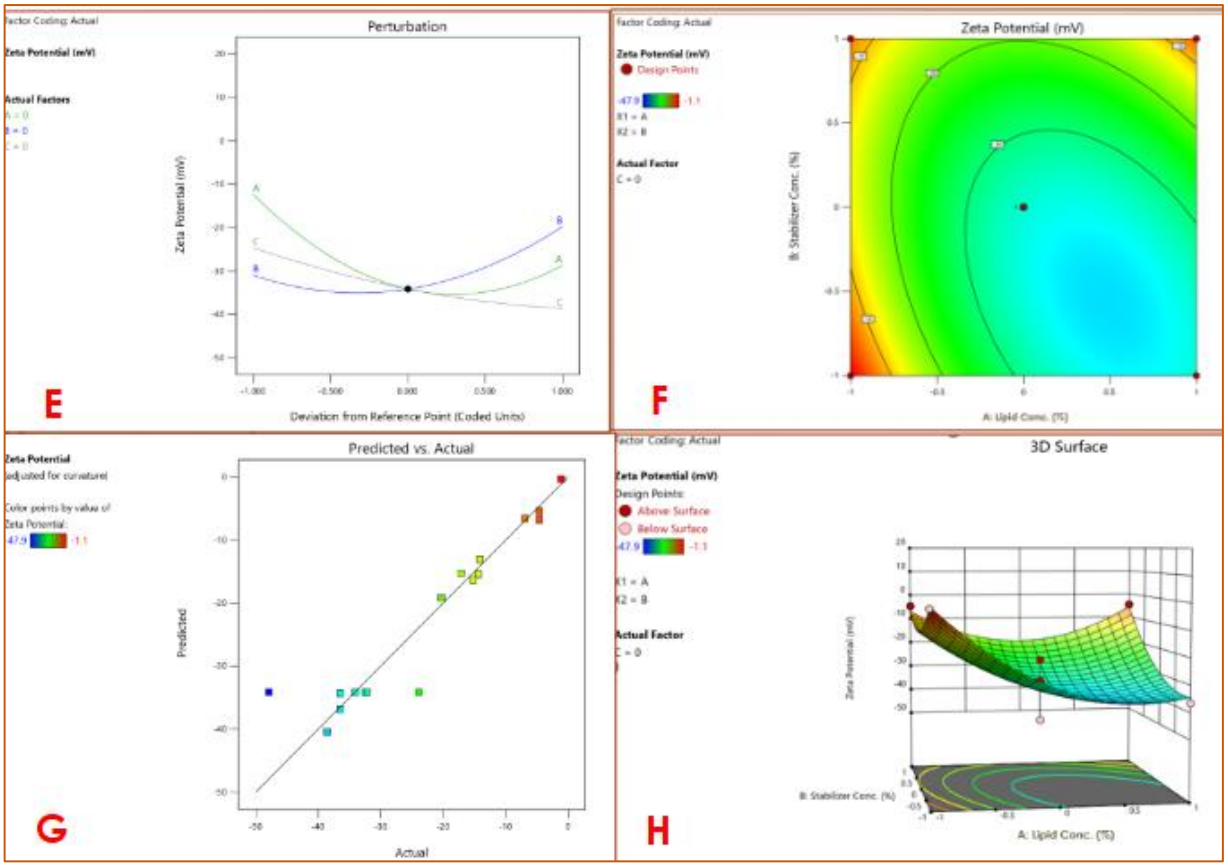

Figure 3: Effect of Variables on Response 2: ZP. E - Perturbation Plot; F - Contour Plot; G - 3D Surface Plot; H - Predicted Vs Actual Value

Light scattering. A tiny aliquot of the dispersion was then diluted ten-fold with deionized water to reduce multiple scattering effects. It was then diluted and placed on a disposable cuvette before analyzing it at 25 degrees Celsius. Mean particle size (z-average) and PDI was recorded with the instrument, which holds a He-Ne laser (633 nm, 10 mW) and a more understanding of the dispersion of the liposome size would be obtained. The measurements have been carried out three times in order to be precise and reproducible[17,18].

Zeta potential

To determine the colloidal stability using the ZETA potential of the liposomal dispersion The HORIBA SZ-100 Zeta Potential Analyzer was used to measure the zeta potential of the liposomal dispersion. The dispersion was then diluted with deionized water (pH 7.4) to obtain optimum conductivity and loaded in a disposable folded capillary cell. The electrophoretic mobility was determined and the Smoluchowski equation used to calculate the zeta potential. In the measure, three readings were taken continuously to find out the average value. Combined values of zeta potential above the critical value of P < 30mV corresponded to good stability which suggests that the effective electrostatic repulsive forces existed between liposomes and aggregation could not take place[19,20].

The Scanning Electron Microscopy

Surface morphology and size distribution of the lyophilized liposomal formulation were determined using Scanning Electron Microscope (JEOL JSM-IT500HR or comparable). Preparing the samples would be by taking a small portion of the formulation and placing it on a block of aluminum using conductive carbon tape on both sides and coating it with gold or platinum using sputter coating for electrical conductivity and contrast development in images. This sample was then introduced into SEM chamber in a high vacuum and the images were taken with varying magnifications at 5-20 kV and imaging was done in a high vacuum. The shape, uniformity and surface of the liposomes were observed using high-resolution images, which are important aspects of both evaluating the morphological aspect of the liposomes[21].

Transmission Electron Microscopy Temperature Led

Analysis was performed on the internal structure, size and morphology of the liposomal formulation tested using a Transmission Electron Microscope (JEOL JEM-2100 or equivalent). Extracting samples To prepare samples, a drop of the liposomal suspension was added on a carbon coated copper grid and left to settle 1-2 minutes. Superfluous sample was diligently eliminated through utilization of filter paper whereas grid remained negatively stained with 1 percent phosphotungstic acid (PTA) or uranyl acetate in addition to elevating relative contrast. The sample-loaded grid was air-dried and inserted into TEM chamber, and the imaging was carried out at accelerating voltage of 80-200 kV. Several images were taken at different magnifications to enable viewing of size, shape, and bilumen of the liposomes. The spherical nature of the morphology, the core-shell plexus and the uniform size distribution were

verified based on the obtained images, which were valuable insights of the nanoscale characteristics on the formulation of liposomes[22].

RESULTS

The UV spectrophotometer scan of the Ropinirole HCl at 287 nm exhibited a linear curve for rise in absorbance against rise in concentration. The values of Absobrance taken at different concentrations (2-10 ug/mL) verified that there is direct correlation, where the formula y = 0.1056x - 0. 0903 and the coefficient of determination (R^2 = 0.9954) was high showing that there was high linear relationship.

The calibration curve is linear indicating that the method proposed by the use of UV spectrophotometry at 287 nm is acceptable and a reproducible method of quantifying Ropinirole HCl. The GA (0.0000) and R^2 (0.9954) values indicate that there is little or no variation against linearity, which means that it is a good method in determining concentration in pharmaceutical constitutions. Through the calibration equation unknown concentrations in samples can be estimated by using their absorbances. The negative intercept (-0.0903) lies very much in an acceptable range showing that the effects of the instrument and the solvent are sluggish. The procedure can be used on regular basis of Quality control, because it is reliable and gives an accurate and reproducible results on the desired concentration range. The quantitative method of Ropinirole HCL by UV spectrophotometry at 287 which shows that the correlation between the concentration and absorbance is strong and has a linear relationship thereby making it a validated method. The calibration equation got is worth using in the estimation of the drug in formulations. The R^2 value is high and it indicates the accuracy and the precision of the method hence it can be used in routine pharmaceutical analysis.

Drug and Excipient Compatibility studies

The Differential Scanning Calorimetry (DSC) examination of pure Ropinirole HCl showed a sharp endotherm at 248 deg C which is its melting point. The physical blend of Ropinirole HCl and the excipients displayed a peak at 249 C only with 1 C difference as compared to the pure form. No considerable spreading, or fading of peaks was noticed. The slightest change in peak temperature indicates that the heat characteristics of the Ropinirole HCl have an insufficient tendency to alter, when there are excipients present. This shows the absence of a large amount of interaction phenomena, that is the drug preserves its crystalline state with no high polymorphic transformations or degradations. In case of the strong interaction, e.g., the formation of complexes or degradation, considerable shifts or broadening of the peaks or their disappearance of the characteristic endotherm of the drug would have been detected. This minor difference in melting points may be explained by some minor physical interactions like hydrogen bonding or minor miscibility with excipients but they are not significant to influence the stability of the drug. DSC analysis has affirmed that Ropinirole HCl is in harmony with the choice of excipients since minimal variation of peak temperature was observed. The lack of major interactions indicates that the excipients will not have any adverse effects to the thermal stability of the drug and

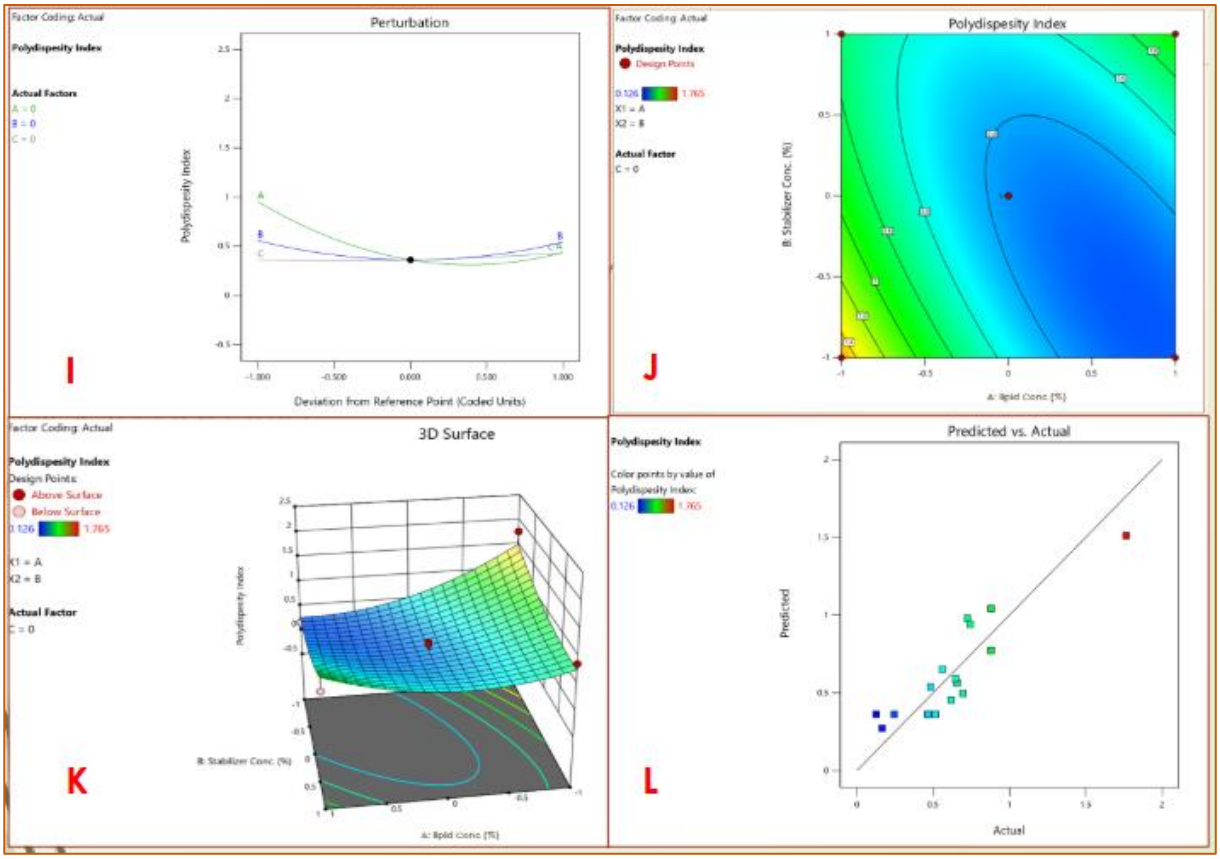

Figure 4: Effect of Variables on Response 3: PDI. I - Perturbation Plot; J - Contour Plot; K - 3D Surface Plot; L - Predicted Vs Actual Value

thus the formulation is satisfactory to be used in further development.

Optimization

The experimental design using the Box-Behnken method evaluated the impact of lipid concentration (phosphatidylcholine), stabilizer concentration (cholesterol), and homogenization speed (RPM) on liposomal particle size, zeta potential, and polydispersity index (PDI).

Particle Size

The smallest particle size was observed in F12 (75.8 nm) at 30% lipid, 5% stabilizer, and 1000 RPM. The largest particle sizes were found in F13 (2345.8 nm) and F11 (2235.5 nm) at low lipid and stabilizer concentrations with lower speeds (500 RPM), indicating inefficient vesicle size reduction. F16 (119.2 nm) and F17 (101.3 nm) also showed relatively small particle sizes, likely due to an optimal combination of lipid and stabilizer levels.

Zeta Potential

The most stable formulation was F15 (-47.9 mV), indicating strong electrostatic repulsion and reduced aggregation. F12 (-38.6 mV) and F6 (-36.5 mV) also showed high stability. F13 (-1.1 mV) and F16 (-4.6 mV) were highly unstable, suggesting possible aggregation due to insufficient charge repulsion.

Polydispersity Index (PDI)

The most uniform formulations were F2 (0.126), F6 (0.165), and F15 (0.244), indicating well-distributed vesicles. F13 (1.765) exhibited the highest PDI, suggesting significant heterogeneity in particle size, which could lead to instability and poor reproducibility.

The results indicate that higher lipid concentrations (30%) generally produced smaller particle sizes due to improved vesicle formation and stability, as seen in F12 (75.8 nm), F16 (119.2 nm), and F8 (146.2 nm). In contrast, formulations with low lipid (10%) and low stabilizer (5%) (such as F13 and F11) resulted in larger, polydisperse particles, suggesting inefficient vesicle formation.

The homogenization speed played a crucial role in controlling particle size. Lower speeds (500 RPM) in F3, F10, and F11 resulted in larger particles (>180 nm), whereas higher speeds (1000–1500 RPM) contributed to smaller and more uniform vesicles.

Zeta potential trends indicate that formulations with moderate lipid and stabilizer concentrations showed better stability, with values above ±30 mV ensuring good electrostatic repulsion. The formulations with extremely low zeta potential (< ±5 mV), such as F13 and F16, indicate poor stability and a higher risk of aggregation.

Polydispersity Index (PDI) data showed that formulations with well-optimized lipid-stabilizer ratios had lower PDI values (<0.3), ensuring homogeneity, while those with imbalanced ratios (e.g., F13: 1.765) exhibited high variability.

The optimal liposomal formulation was found to be F12 (75.8 nm, -38.6 mV, 0.559 PDI) due to its small particle size, good stability, and moderate uniformity, making it the most suitable candidate for further pharmaceutical applications. F15 (-47.9 mV, 187.4 nm, 0.244 PDI) also demonstrated excellent stability with a lower PDI, making it another strong candidate.

In contrast, formulations such as F13 (2345.8 nm, -1.1 mV, 1.765 PDI) and F11 (2235.5 nm, -6.9 mV, 0.742 PDI) were highly unstable due to large particle sizes, poor zeta potential, and high polydispersity. These findings confirm that lipid and stabilizer concentration, along with homogenization speed, must be carefully optimized to develop stable and uniform liposomal formulations of Ropinirole HCl.

Response 1: Particle Size

The ANOVA analysis of particle size optimizing showed a significant model (F-value = 23.88, p- value = 0.0002), which implies that the selected factors facilitate the variability in particle size. It was noted that:

The greatest impact on particle size was exerted by Lipid Concentration (A) with both its linear ($p < 0.0001$) and squared ($p < 0.0001$) terms as it suggests that rise in lipid concentration greatly increases the particle size.

The linear term of Homogenization Speed (C) was also vital ($p = 0.0326$) and this represented that the increase in the speed translated into smaller particle size.

There was interaction between the concentration of Lipid and the speed of Homogenization (AC) ($p = 0.0316$) and this indicated that the effect of lipid concentration on the particle size is dependent on homogenization speed.

Non-Significant Factors

There was no significant difference in terms of surge crystal size with increasing levels of Stabilizer Concentration (B) ($p = 0.4631$), which means that the level of cholesterol concentration in the range of 5-15 percent had no pronounced effect.

The terms, i.e., the interactions AB (Lipid x Stabilizer), BC (Stabilizer x Speed), and B 2 (Quadratic Stabilizer Effect), were not significant ($p > 0.1$) that strengthens the evidence that the stabilizer concentration concentration is not a critical factor in predicting particle size.

According to the results, concentrations of lipid and the rates of homogenization of the liposomal formulation of Ropinirole HCl are the main factors defining the size of the particle. Higher availability of phospholipids forming large vesicles has a significant effect of increasing the size of the particles owing to the higher concentration of lipids. Conversely, a faster speed of homogenization produces smaller sized particles because there is more shear force that causes the larger-sized vesicles to break into smaller ones.

The high interaction between lipid concentration and homogenization speed (AC) denotes the effect of association of lipid concentration and particle size is sectional upon homogenization speed. The rise in the lipid concentration could be of less noticeable influence at faster rates of homogenization since the shear force influences reduce the size of the particles. On the other hand, lipid concentration assumes a significant role at lower speeds which define much bigger vesicles.

Stabilizer concentration (cholesterol) also did not considerably affect particle size implying that the phospholipid to cholesterol ratio had no significant effect on the formation of vesicles in the tested range (5-15%). The best formulation will be to strike the compromise

between the quantity of lipids and the rate of homogenization to attain the desired stable and small particle size. Growing concentration of lipid in the solution results in a bigger particle size and vice versa, accelerating the process of homogenization causes the size of particles to go down.

The most favorable circumstances of smaller particle size would be low concentration of lipids and high homogenization rate which is in F12 (75.8 nm, -38.6 mV, 0.559 PDI) and F16 (119.2 nm, -4.6 mV, 0.724 PDI). Lipid / low-speed combinations: High lipid and low-speed combinations (F13, F11) produced highly large particles (>2000 nm) and did not exhibit good stability, hence were not suitable to formulate. The insignificance of stabilizer concentration means that at the levels that have been tests, minimizing of lipid concentration and rate is more imperative as compared to manipulating cholesterol levels. This demonstrates that the concentration of lipid and the rate of homogenization must be put under consideration of priority when optimizing the process of formulation of Ropinirole HCl through smaller, stable, and uniform liposomes.

Response 2: Zeta Potential

ANOVA analysis of Zeta Potential indicated that the model was significant ($F = 6.90$, $p = 0.0093$) which implies that the variables thus chosen give an effective explanation to the variation in Zeta Potential. The main results are the following:

Respondent

Lipid Concentration (A): Significant ($F = 11.54$, $p = 0.0115$), which means that quite an impact on Zeta Potential is observed with regards to lipid concentration.

Lipid Concentration Quadratic Term (A2): Extremely significant ($F = 16.92$, $p = 0.0045$) pointing to the fact that there is a nonlinear relationship between the lipid concentration and the Zeta Potential with the potential that above a certain level of lipid concentration, the stability level may fail to increase in proportion to the increase in the concentration of the lipid.

Correlation of Lipid Concentration and Stabilizer (AB): Specific ($F = 6.78$, $p = 0.0352$), which means that the interaction of lipid concentration and stabilizer concentration has a more significant effect on Zeta Potential than spaced-out components.

Moderate Significant Factor

Stabilizer Concentration (B): Marginally significant ($F = 5.43$, $p = 0.0526$), that is, it can slightly influence Zeta Potential, but not as well as the lipid concentration.

Non- Significant Factors

Homogenization Speed (C) and its interactions (AC, BC, C2): Not significant (p1: Homogenization Speed (C) and its interactions (AC, BC, C2): Not significant ($p > 0.1$), indicating that the speed does not affect Zeta Potential significantly.

The findings have revealed that lipid concentration (A) is the most influential variable that is determining Zeta Potential showing a significant linear and quadratic effect. At first, then, as the level of lipid increases, Zeta Potential is upgraded because the electric charge at the surface of the vesicle becomes more impressive, which induces greater electrostatic repulsion as well as stability. Nevertheless, the quadratic nature of the significance implies that above some threshold level of lipids, an increase in the concentration might not be proportional to the stability gain, which may be caused by charge saturation or by vesicle aggregate formation.

Lipid concentration and stabilizer (AB) were also explained by the interaction, which indicates that there needs to be the right proportion between lipid and stabilizer in obtaining stable liposomes. Membrane rigidity is assisted by higher concentrations of the stabilizers (cholesterol) that in turn affect the surface charge. Nevertheless, its influence was of a slight significance, which means that the concentration of lipids was more prone to influence Zeta Potential in comparison with stabilizer concentration in full range of its concentration (5 15 percent).

The insignificance of the speed of homogenization (C) and its interaction implies that the shear forces influence the size of the particles only, but are not a key factor in modifying the surface charge (Zeta Potential).

The most important factor that influences the Zeta Potential and with a strong non-linear influence is lipid concentration (A). Stability increases as the concentration of lipid increases up to a certain point and it may reach equilibrium point above an optimal level.

The interaction between lipid and stabilizer concentration (AB) is significant i.e. there has to be a particular ratio between lipid and stabilizer in order to achieve optimum Zeta Potential.

The concentration of stabilizer (B) moderately affects charge stability, and it implies that a refined concentration of cholesterol can marginally enhance charge stability, but is not as significant as the percentage of lipid.

That is, C does not play an important role in Zeta Potential which means that the charge stability of the liposomes will be more affected by the formulation composition than the processing speed.

Comparison to other Data

The findings are consistent with optimization of the production of particles where the concentration of lipid also played an important role further validating its dual attributes in the production and stability of vesicles.

Whereas in the case of the particle size, homogenization speed was a key factor, Zeta Potential was also independent of speed, once again confirming that charge stability is less about the process of mechanical dispersion of liposomes than about their composition.

The best formulation will be the one that maintains the concentration of the lipid and stabilizer to reach the particular dimensions of the sizes of the particle (small) and high Zeta Potential ($> \pm 30$ mV) to guarantee physical stability and avoid aggregation.

To obtain a stable biochemistry of liposomal formulations in Ropinirole HCl the lipid concentrations should be optimized at a careful level so as to increase Zeta Potential and there should be a balance between the lipid concentration and the stabilizer concentration. Other processing parameters that do not affect surface charge are important in particle size control i.e. homogenization speed. The results affirm that an ensured optimal lipid to stabilizer

Table 3: Optimization of Liposome

Formulation	Lipid Concentration (%) (Phosphotidyl Choline)		Stabilizer Conc. – (Cholesterol) (%)		Homogenization Speed (RPM)		Particle Size (nm)	Zeta Potential (mV)	Polydisper -sity Index
	Level	Conc.%	Level	Conc.%	Level	RPM			
F1	0	20	0	10	0	1500	190.7	-32.2	0.467
F2	0	20	0	10	0	1500	193.2	-23.9	0.126
F3	0	20	-1	5	-1	500	411.6	-15.2	0.482
F4	0	20	0	10	0	1500	196.5	-32.5	0.514
F5	0	20	0	10	0	1500	195.8	-34.1	0.461
F6	1	30	-1	5	0	1500	328.4	-36.5	0.165
F7	-1	10	1	15	0	1500	2122.2	-4.6	0.879
F8	1	30	0	10	1	1000	146.2	-36.5	0.695
F9	0	20	1	15	1	1000	233.6	-20.3	0.647
F10	1	30	0	10	-1	500	182.7	-14.4	0.617
F11	-1	10	0	10	-1	500	2235.5	-6.9	0.742
F12	0	30	-1	5	1	1000	75.8	-38.6	0.559
F13	-1	10	-1	5	0	1500	2345.8	-1.1	1.765
F14	-1	10	0	10	1	1000	1014.2	-14.1	0.879
F15	0	20	0	10	0	1500	187.4	-47.9	0.244
F16	1	30	1	15	0	1500	119.2	-4.6	0.724
F17	0	20	1	15	-1	500	101.3	-17.1	0.656

ratio is decisive to attaining not only a low nanoscale dimension of particles but also high values of Zeta Potential in order to enhance liposome stability.

Response 3: Polydispersity Index

The ANOVA on Polydispersity Index (PDI) yielded that the model is somewhat significant (F-value = 3.20, p-value = 0.0496) which means that although the model can explain some variability in PDI it is not as predictive as the particle size and the zeta potential models. The most important observations are the following ones:

Major aspects

There was significant effect of Lipid Concentration (A) (F = 8.85, p = 0.0206), which means that any alteration in lipid concentration may affect the distribution of particle size, which is likely to interfere with uniformity of the liposomes.

Appreciable interaction was also noticed between the Lipid Concentration and Stabilizer Concentration (AB) (F = 8.68 and p = 0.0215), which means that there should be an equilibrium of lipid and stabilizer concentration to maintain the PDI.

The quadratic term of the lipid concentration (A^2) was also found to be significant (F = 7.81, p = 0.0267) which translates that there is no linear relationship between the level of lipid and the PDI, i.e., above some value of lipid concentration, an increased value of lipid would not necessarily enhance uniformity.

Non-Significant Factors

Stabilizer Concentration (B) and Homogenization Speed (C) were not significant (p = 0.9280 and p = 0.6955 respectively) which is an indication that between tested limits (5-15% cholesterol and 500-1500 RPM), there is insignificant effect on the PDI by these two variables.

Other interactions (BC, AC and C^2) were also not significant (p > 0.1), which empowers the idea that homogenization speed and stabilizer concentration do not have a statistically significant contribution to PDI.

The findings indicate that the main determinant of PDI is the lipid concentration, as was the case with the particle size and the zeta potential. To a certain extent, increased lipid concentration leads to better distribution lessening PDI. Nevertheless, the relevance of the quadratic term (A^2) implies that above an optimum lipid concentration,

Table 4: ANOVA of Particle size shows significant effect of variable on particle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.051E+07	9	1.167E+06	23.88	0.0002	significant
A-Lipid Conc.	6.554E+06	1	6.554E+06	134.08	< 0.0001	
B-Stabilizer Conc.	29439.51	1	29439.51	0.6022	0.4631	
C-Hom.Speed	3.450E+05	1	3.450E+05	7.06	0.0326	
AB	24711.84	1	24711.84	0.5055	0.5001	
AC	3.509E+05	1	3.509E+05	7.18	0.0316	
BC	17969.40	1	17969.40	0.3676	0.5635	
A^2	3.043E+06	1	3.043E+06	62.25	< 0.0001	
B^2	51926.98	1	51926.98	1.06	0.3370	
C^2	92473.68	1	92473.68	1.89	0.2114	
Residual	3.422E+05	7	48882.64			
Lack of Fit	3.421E+05	3	1.140E+05	8089.71	0.6871	Nonsignificant
Pure Error	56.39	4	14.10			
Cor Total	1.085E+07	16				

Table 5: ANOVA of Particle size shows significant effect of variable on Zeta potential

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2869.00	9	318.78	6.90	0.0093	significant
A-Lipid Conc.	533.01	1	533.01	11.54	0.0115	
B-Stabilizer Conc.	250.88	1	250.88	5.43	0.0526	
C-Speed	390.60	1	390.60	8.46	0.0227	
AB	313.29	1	313.29	6.78	0.0352	
AC	55.50	1	55.50	1.20	0.3093	
BC	102.01	1	102.01	2.21	0.1808	
A^2	781.36	1	781.36	16.92	0.0045	
B^2	325.88	1	325.88	7.05	0.0327	
C^2	26.79	1	26.79	0.5800	0.4712	
Residual	323.34	7	46.19			
Lack of Fit	22.69	3	7.56	0.1006	0.9555	not significant
Pure Error	300.65	4	75.16			
Cor Total	3192.34	16				

Table 6: ANOVA of Particle size shows significant effect of variable on Polydispersity index

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.73	9	0.1925	3.20	0.0496	Significant
A-Lipid Conc.	0.5325	1	0.5325	8.85	0.0206	
B-Stabilizer Conc.	0.0005	1	0.0005	0.0088	0.9280	
C-Speed	0.0100	1	0.0100	0.1665	0.6955	
AB	0.5220	1	0.5220	8.68	0.0215	
AC	0.0009	1	0.0009	0.0145	0.9076	
BC	0.0018	1	0.0018	0.0307	0.8658	
A^2	0.4699	1	0.4699	7.81	0.0267	
B^2	0.1469	1	0.1469	2.44	0.1620	
C^2	0.0057	1	0.0057	0.0948	0.7671	
Residual	0.4210	7	0.0601			
Lack of Fit	0.3074	3	0.1025	3.61	0.1234	not significant
Pure Error	0.1135	4	0.0284			
Cor Total	2.15	16				

additional changes will result in polydisperse vesicles because of the excess amount of aggregated lipids.

The influence of the combination of the lipid concentration and stabilizer concentration (AB) was also significant, and that means the amount of lipid to stabilizer ratio is essential in obtaining uniform distribution of vesicle size. Excessive or insufficient stabilizer with respect to lipid content might result in vesicle fusion, or particles of non-uniform size and hence higher PDI.

Concentration of stabilizers (B) did not reveal any significant influence on PDI either, so concentration of cholesterol did not significantly influence the size distribution control at the level of concentration tested (5 to 15%). In the same vein, the speed of homogenization (C) was not significant indicating that although speed is vital in decreasing all round particle size, it does not affect significantly the homogeneity of size distribution (PDI).

The lipid concentration (A) is the most critical parameter affecting PDI, and this proves it to be the most central when it comes to liposomal formation and stability.

A good balance between lipids and stabilizer must be obtained as it is effective in the case of the strong AB interaction, where vesicles of homogenous distribution are achieved.

An optimum lipid state will be followed by grater values of PDI in case of an increase beyond this point, an idea indicated by the strong quadratic effect (A^2).

Stabilizer concentration (B) and the homogenization speed (C) do not have a profound influence on PDI, which would indicate that a vesicle size distribution depends more on a formulation composition than processing factors.

Comparison to Other data

Particle size optimization: The concentration of lipid and the speed of homogenization played an important role whereas the concentration of stabilizers did not. This is correlated with PDI results, whereby, the lipid concentration was significant, and speed and stabilizer did not make a significant impact.

Optimization of zeta potential: The main factor was the concentration of lipid, and its quadratic effect (A^2) was pronounced. This is what the PDI shows, which infers that the lipid levels also determine the uniformity of the vesicle, as well as, the stability of surface charges.

As opposed to the particle size, where homogenization speed had crucial role, the speed also did not have significant influence on PDI, proving once again that size reduction does not always result in evenly dispersed vesicles.

In order to obtain low PDI (more representative vesicle size distribution) in Ropinirole HCl Liposomal formulations, it is recommended that manipulation of lipid concentration be carried out thoroughly. Although the rate of homogenization decreases the size of the particles, the effect on the level of uniformity is not observed and the

concentration of a stabilizer does not influence the PDI as well. This is most effectively achieved by ensuring that there is an equilibrium of lipid and stabilizer concentrations so that size related variation is reduced and formulation robustness increased.

Polynomial Equations

Particle Size=192.72−905.15A−60.6625B−207.663C+78.6AB+296.2AC+67.025BC+850.128A2−148.198C2

The concentration of lipids (A), the speed of homogenization (C), their interaction (AB, AC) and the squared concentration of lipids (A^2) heavily influence the value of the particle size. As the equation of the particle size demonstrates, the most significant impact on particle size is made by the lipid concentration (A), the quadratic and linear parts of its influence play a significant role in the particle dimension as well. The response is monotonic because as the lipid concentration rises the particles will also increase in size and the significance of quadratic term (A^2) is an indication that at a point of high lipid concentration the size will then grow exponentially because of aggregation of the lipids. The speed of homogenization (C) is also very important in minimizing particle size whereby the use of higher speeds produces smaller vesicles since they increase the shear forces. Interactions were found to be significant between the lipid concentration and stabilizer (AB) and this indicates that the presence of lipid and cholesterol may have an effect in the size of the particles. Also the interaction between the lipid concentration and speed (AC) proves that the effects of lipid concentration is controlled by the speed of homogenization (so that the effects of the concentration of lipid on size might be attenuated at greater speeds). Worth noting is that the concentration of the stabilizer (B) as such has little effect on the size of the particles, implying that up to the tested range (515%), the addition of cholesterol does not apparently affect vesicle sizes to a significant degree. According to these results, it is important to optimize the drive of the concentration and the rate at which the lipids are homogenized to obtain an acceptable particle size in liposomal formulation.

Zeta Potential=−34.12−8.1625A+5.6B−6.9875C+8.85AB−3.725AC+5.05BC+13.6225A2+8.7975B2+2.5225C2

The zeta potential is significantly dependent on Lipid concentration (A), stabilizer concentration (B), their interactions (AB) and square of Lipid concentration (A 2). Zeta potential equation points out that the concentration of lipid (A) is critical in the determination of the stability of the surface charges, the linear and quadrate terms of the equation are vital. The quadratic effect (A 2) implies that the effect is not linear, i.e. a proportional change in ZP is not observed as the concentration of lipid is raised. The concentration of stabilizer (B) influences the zeta potential much more than the particle size of the object under study since cholesterol, in this case, will make the membrane rigid and influence the factor of overall surface charge. The speed of homogenization (C) exerts a moderate effect, thus showing that the processing conditions may modify the surface charge distribution but are not the main factor of determining stability. Of the meaningful interactions, AB (Lipid x Stabilizer) indicates clearly that the combined effect of lipid and cholesterol is a very relevant determinant of zeta potential so that the concentration of a stabilizer should also be optimized along with interaction concentration so that a high electrostatic stability is achieved. The high value of A 2 (Lipid 2) and B 2 (Stabilizer 2) reveals that both of concentration of lipid and stabilizer show non-linear influence of zeta potential and its effect may saturate or cross over to another effect at high concentration. This discovery indicates that it is important to have a perfect balance between lipid and stabilizer concentration to create an optimum zeta potential to make stable liposomal products.

Polydispersity Index=0.3624−0.258A−0.008125B+0.035375C+0.36125AB−0.01475AC−0.0215BC+0.33405A2+0.1868B2+0.0368C2

Lipid concentration (A) and squared term (A produce the most dominant effect on polydispersity index although that of stabilizer concentration (B) and the speed of homogenization (C) have limited importance in the entire range studied. Equation of polydispersity index (PDI) reveals that the concentration of lipid (A) has the greatest impact on the consistency of the vesicle indicating that the most important factor that shows whether the PDI will be high or low is the lipid composition of the formulation. The quadratic term (A^2) is important implying that, when the lipid concentration is > an optimal level, high polydispersity occurs which can be due to aggregation of lipids forming non regular vesicles. The relation between lipid concentration and stabilizer concentration (AB) is also important implying that there is a good balance needed between lipid to stabilizer concentration needed to provide uniformity in the distribution of vesicles. Nevertheless, homogenization speed (C) and stabilizer concentration (B) do not make a crucial direct influence on PDI, which proves that processing conditions matter less than a formulation composition in terms of vesicle uniformity. These results underline that the best results in terms of PDI of the resulting formulation are achieved when optimizing the lipid concentration and its synergism to the stabilizer concentration, to obtain a low PDI and keep the formed vesicles consistent and stable.

The optimization of liposomal formulation of Ropinirole HCl brings out the concentration of lipids (A) as the most significant that contributes to all the three most vital parameters; namely particle size, zeta potential, and polydispersity index (PDI). Doubling the amount of lipid roughly triples the vesicle size whereas its quadratic term (A^2) implies that the values of the excess lipid concentration cause aggregation and the lack of uniformity. Lipid concentration also influences the zeta potential, stabilizer concentration (B) follows up in ensuring the stability of the charge. The rate of homogenization (C), though, plays a significant role in the reduction of the particle sizes but does not have a big role to play in zeta potential or PDI.

To optimize the liposomal formulation, the size of the particle can be reduced by raising homogenization rate to at least 1500 RPM, but keeping the lipid concentration at moderate level to discourage vesicle enlargement. A balanced lipid to stabilizer ratio is important and unnecessarily high lipid levels can make the charge unstable (zeta potential > 330 mV). In order to attain the

desired low PDI and monodisperse vesicles, the lipid concentration is to be kept in careful check and the optimal interaction between the lipid and the stabilizer concentrations should be pursued.

The models shown by the police have confirmed that there is indeed no doubt that the most significant variable in this is the concentration of lipids, the speed of homogenization in size reduction, and the concentration of stabilizers in charge stability. Such models can be useful predictive models in designing of small stable and uniformly distributed liposomal vesicles of Ropinirole HCl to enhance the formulation performance and stability.

Optimized liposomal formulation (F12) was made under the following conditions: 30% phosphatidylcholine as a lipid concentration, 5% cholesterol as a stabilizer concentration, and 1000 RPM as a speed of homogenization. These settings gave a particle size of 75.8-nm, zeta potential of -38. 6mV, and a polydispersity index (PDI) of 0.559 with a small, stable, and moderately uniform particle distribution. It is affirmed by the optimization that concentration of lipid is the most important variable affecting all the three responses and the speed of homogenization is important in minimizing the size of particles. The concentration of the stabilizer is also used to provide stability in charge and also suppresses aggregation. Such results confirm that F12 is the best formulation that represents the efficacy of F12 in increasing drug delivery efficiency as well as stability and therapeutic efficiency of such liposomal Ropinirole HCl formulations.

DISCUSSION

A dopamine D2/D3 receptor agonist, Ropinirole HCl, which has already found its application in the neuron-related conditions, attracted the attention recently, because of its possible anti-cancer effects. Its working formula of causing cancer cells in dopamine receptor to die (apoptosis) and inhibit cancer cells growth (angiogenesis) makes it an enviable candidate in breast cancer treatment. Nonetheless, it has been limited in its clinical use in oncology by limited solubility and rapid systematic clearance with low bioavailability. These limitations have been effectively overcome by the study by using Ropinirole HCl in a liposomal delivery system as it has the ability to increase the solubility and target specificity as well as therapeutic efficiency[1-4].

There is systematic optimization of liposomal formulation in Quality by Design (QbD) approach employed in this research. On examining the impacts of some of the variables, all variables were involved, which included lipid concentration, stabilizer concentration, and speed of homogenization, but the lipid concentration proved to be the critical factor that affected the size of the particle, zeta potential and polydispersity index (PDI). Increased lipid levels were more likely to contribute to augmenting the sizes and the zeta potential of the particle, where extreme concentrations led to aggregation of the vesicles and augmenting the PDI. The rate of homogenization was a crucial factor when determining the particle size and had no significant effect on zeta potential or PDI, therefore, the adjustment of formulation variables should be a priority toward the preparation of a stable and homogeneous liposomal system[5-8].

An optimum formulation (F12) generated by Box-Behnken Design showed a perfect particle size of 75.8 nm, zeta potential -38.6 mV and PDI of 0.559. These values represent great colloidal stability, good tumor tissue permeation, and average uniformity. The low particle size is improved to promote passive targeting through the enhanced permeability and retention (EPR) effect and the high negative charge on the surface diminishes aggregation. The more consistent drug release profile is also achieved with this formulation, which proposes the formulation to be used as a targeted treatment against breast cancer. The prepared formulations of F12 were observed to be stable and perform better than the other formulations that did not show any good size control and also poor zeta potential, particularly F13 and F11[9-14].

Such findings are consistent with the available literature on liposomal drug therapy in cancer since it proves once again that the choice of lipid is a critical first step towards the desired vesicle features. Thus, similarity liposomal systems tend to support the implication of regulating the lipid-to-cholesterol balances and processing parameters in generating the nanosize vesicle formulating stable surface charges. The findings also re-stress the importance of the fact that the sole influence of the stabilizer concentration (cholesterol) on particle size and PDI is secondary, whereas charge stabilization is the main influence of stabilizers. The strong predictive models developed in this paper provides a basis of scaling up the formulations and modifying it to suit other hydrophobic therapeutic compounds[15-21].

The study presented here does not only demonstrate an effective liposomal stable platform to Ropinirole HCl but draws attention to the repurposing of drugs in cancer treatment as well. The liposomal system raises the treatment benefit and diminishes the systemic toxicity of the treatment, a typical drawback of the traditional treatment regimens in cancer chemotherapy, as a result of a better pharmacokinetic profile and delivery specificity. Qualitative validation of clinical translatability should accomplished by conducting in vitro cytotoxicity studies, biodistribution testing, and in vivo efficacy studies in the future. The incorporated QbD will guarantee the reliability of formulation reproduction and scaling, which will allow taking into account its usage in clinical practice as a method of managing breast cancer.

CONCLUSION

The study reveals the possibility of the use of liposomal Ropinirole HCl as a practical treatment method of breast cancer since it complements the shortcomings of regular chemotherapy. Optimized formulation (F12) demonstrated a particle size of 75.8 nm, a zeta potential of -38.6 mV and polydispersity index value of 0.559, which are indications of increased stability and effectiveness in targeted drugs delivery. It is also ascertained that the concentration of lipid, stabilizer and the speed of homogenization has significant effects on the physicochemical properties of the formulation. Not only does the incorporation of QbD guarantee reproducibility and scalability, but so also does

the encapsulation in liposomes increase bioavailability, minimize the scan of systemic toxicity and extends the life of drugs. Additional in vitro and in vivo experiments should be done to confirm its therapeutic potential, pharmacokinetics and biodistribution, which will open the prospect of its clinical use in treatment of breast cancer.

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