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FORMULATION AND *IN-VITRO* CHARACTERIZATION OF APREPITANT LOADED LIPID NANOPARTICLES WITH FACTORIAL DESIGN-BASED STUDIES USING QUALITY BY DESIGN (QbD) APPROACH

PANDA CHINTAMANI[1*], CHAUHAN SACHINKUMAR PRABHUBHAI[2] AND BALAMURUGAN KRISHNAN[1]

1: Department of Pharmacy, Annamalai University, Faculty of Engineering and Technology, Annamalai Nagar, Chidambaram, India

2: Formulation Development, Navin Saxena Research and Technology Pvt. Ltd., Gandhidham, Gujarat

***Corresponding Author: Chintamani Panda: E-Mail: chintamanipanda@gmail.com, placementbala@yahoo.co.in**

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ABSTRACT

Aprepitant (AP) is a selective high-affinity antagonist of human substance P/neurokinin 1 (NK1) receptors that is used for the prevention of acute and delayed nausea and vomiting caused by initial and repeat courses of highly emetogenic cancer chemotherapy. It has low aqueous solubility that delays the absorption from the gastrointestinal tract (GIT) when administered through oral route. Solubility parameter of a drug substance is one of the key factor of its oral bioavailability and low oral bioavailability requires development of drug delivery systems able to improve its solubility and bypass hepatic effect. The purpose of the present research paper was to investigate the possibility to encapsulate Aprepitant into nanostructured lipid carriers (NLCs) to improve the drug release and in long run oral bioavailability by using of Quality by Design (QbD) approach for the design, development, optimization and characterization of nanostructured lipid carriers containing Aprepitant (AP-NLCs). For this purpose, AP-NLCs were fabricated by emulsification using high-speed homogenization followed by ultrasonication. The 2^3 full factorial plan was utilized to assess the connection between the Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) variable. The most basic critical quality attributes (CQAs) studied were particle size analysis and entrapment efficiency. The particle size

of prepared NLCs were found to be between 179.2 - 485.5 nm while zeta potential values varied between 40.02 ± 0.24 and 15.42 ± 0.31 mV. They also showed high encapsulation efficiency (>80%) and amorphous state of the drug in lipid matrix.

Keywords: Aprepitant, Nanostructured Lipid Carriers (NLCs), Ultrasonication, Quality by Design (QbD), factorial design

INTRODUCTION

Chemotherapy-induced nausea and vomiting (CINV) are two of the most common and worrisome side effects experienced by cancer patients. Aprepitant (AP) is a selective high-affinity antagonist of human substance P/neurokinin 1 (NK1) receptors that is indicated for the prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of highly emetogenic cancer chemotherapy **[1]**.

The aqueous solubility of Aprepitant is 3–7 µg/mL (very low) over the 2-10 pH range **[2]**. Because of its very low aqueous solubility, it is challanging to develop a formulation using conventional approaches that will provide adequate systemic exposure to produce a therapeutic effect. The currently marketed formulation of aprepitant (Emend®) is based on nanoparticle technology in which its solubility is increased using drug nanoparticles. The complex nature of nanoparticle technology in terms of processing and effort required necessitate exploration of alternate technologies for solubility enhancement **[3-5]**.

Lipid-based drug delivery systems are promising drug carriers due to their ability to improve solubility of poorly water-soluble and/or lipophilic drugs which eventually enhance the oral bioavailability **[6,7]**. Nanostructure lipid carriers (NLCs), the second generation of lipidic nanoparticles, has the advantage over the first generation of lipidic nanoparticles, the so-called solid lipid nanoparticles (SLNs) as it provides a higher payload and prevents drug expulsion during storage. Higher drug loading is attributed to the differences in the chemical structure between liquid and solid lipids, which result in distortion of a perfect crystal and accommodation of drug in molecular form or in amorphous clusters **[8]**.

The objective of the present study was to explore the feasibility to encapsulate aprepitant into NLCs to improve dissolution rate and eventually oral bioavailability. The nanoparticulate formulations were prepared by emulsification using high-speed homogenization followed by ultrasonication process. A 2^3 full factorial design was

employed. Experimental variables such as ratio of solid lipid/liquid lipid concentration were varied to study their effect on drug entrapment efficiency and release rates of drug from nanoparticles. Glyceryl Behenate (Compritol 888 ATO) were chosen as solid lipids while Caprylocaproyl polyglycerides (Dubcare GPE 810) was selected as liquid lipid. Polysorbate 80 was used as hydrophilic surfactants, while lecithin was selected as lipophilic emulsifier. NLCs were optimized for physicochemical properties.

Materials

The API (Apripitant), Glyceryl Behenate (Compritol 888 ATO), Caprylocaproyl polyglycerides (Dubcare GPE 810), MCT (Stelliesters MCT 65/35) Lecithin (Lipoid S20), Silicon Dioxide (Syloid XDP 3150) and Polysorbate 80 (Tween 80) were received as gift sample from Cadila Pharmaceuticals Ltd., Ahmedabad, . All other chemical used were of analytical grade.

Methods

Preformulation Study

Saturated solubility studies

The saturated solubility studies were performed to determine the solubility of drug in the components to be used in the formulation such as sold lipid, liquid lipid and surfactants. For solid lipid known amount of drug was added to measured quantity of lipid. The minimum amount of melted lipid required to solublize the drug was noted visually within 24 hours. The end point of the solubility study was the formation of clear solution of molten lipid.

Compatibility study of solid and liquid lipid

The solid lipid to liquid lipid ratio of 7:3, 5:5 and 3: 7 were studied. The mixtures selected were stearic acid: Medium Chain Triglycerides, Glyceryl behenate: Caprylocaproyl polyglycerides, and Glyceryl monostearate: Linseed oil. Each mixture were taken in the above three mentioned ratios. Mixtures were heated up to 5 °C more than the melting points of the solid lipid and then checked after 1 hour, immediately after solidification and then after 24 h. Mixtures with only one single phase were selected for further study.

Drug polymer interaction study

To verify the purity of the obtained samples like drug and excipients, FTIR spectra were verified (FTIR BRUKER) over a wavelength range of 4000-400 cm^{-1} at resolutions of 6 cm^{-1}. Samples were directly place on the probe and spectra were recorded. The FTIR spectra of pure Aprepitant, and mixture of all the ingredients were recorded to check drug polymer interaction and stability of drug.

Formulation Studies

Preparation of AP-NLCs

The Aprepitant (AP) loaded NLCs were prepared by emulsification using high-speed homogenization followed by ultrasonication. The lipid and aqueous phases were prepared separately. Lipid phase consisted of solid lipid (Glyceryl Behenate), liquid lipid (Caprylocaproyl polyglycerides) and lipophilic emulsifier, while the aqueous phase consisted of hydrophilic emulsifier (Tween 80) dissolved in distilled water. AP was dissolved in Caprylocaproyl polyglycerides and then mixed with other lipid phase components. All components of lipid phase were heated separately to 10°C above solid lipid transition temperatures (58–60°C) for 10 min before mixing. The aqueous phase was added drop-wise to the molten lipid phase and mixed using a high-speed homogenizer (Janke & Kunkel, GmbH, Staufen, Germany) at 10,000 rpm for 10 min. The mixture was further treated using a probe-type sonicator (ultrasonic processor, GE130, probe CV18, Newtown, CT). The resultant emulsions were adsorbed on to the granular colloidal silicone dioxide and cooled at room temperature. The NLC adsorbed powder was filled into capsules.

Formulation Optimization of AP-NLCs as per Design of Experiments (DoE)

The development of a formulation that meets the desired CQAs is the ultimate goal of all formulation development activities. The formulation components of AP-NLCs that have a high potential to impact the drug product CQAs are: the solid lipid/liquid lipid ratio, the surfactant concentration (polysorbate 80) and the ultrasonication time. The water quantity, the temperature has a low to medium risk to impact the CQAs. As Aprepitant is a nanoparticulate formulation, particle size is the most critical quality attributes to study. In addition to this the encapsulation efficiency (%) of the Aprepitant in nanostructured lipid particles is also considered as the dependent variable.

Therefore, particle size and entrapment efficiency (%) were selected as an output response. Hence, to achieve optimum performance, the formulation design space has to be established within which all the CQAs are met.

In order to achieve this, a 2^3 full factorial design with 3 center point study was used to optimize the levels of solid/liquid lipid ratio, Surfactant concentration and ultrasonication time to establish the formulation and process space (**Table 1**).

Statistical analysis was conducted using the software Design Expert 7.0. (trial version). The trials were conducted in a random

fashion in the order given by the software in order to avoid any intentional error. An overview of different formulations prepared by changing the ratio of solid/liquid lipids, surfactant concentration and ultrasonication time is outlined in below **Table 2, 3**.

Table 1: Design of the 2^3 full factorial design with 3 center point DoE for AP-NLCs

Factors : Critical Formulation and Process Variables		Levels		
		-1	0	+1
A	Solid Lipid /Liquid Lipid Ratio (X_1)	3:7	5:5	7:3
B	Surfactant Concentration (%) (X_2)	5	7.5	10
C	Ultrasonication Time (min) (X_3)	10	15	20
	Responses	Goal	Acceptance Criteria	
Y1	Particle Size (Y_1)	Minimize	NMT 250 nm	
Y2	Entrapment Efficiency (%EE) (Y_2)	Maximize	To achieve as maximum as 100%	

No. of factors = 3, No. of levels = 2, Replicates = 0, No. of centre points = 3; No. of experimental runs = (2 x 2 x 2) + 3 = 11 runs

Table 2: Experimental Results of the DOE to Study Critical Formulation Variables and Critical Process Parameter for AP-NLCs

Run No.	Batch No.	Type	X_1: Ratio of solid/ liquid lipid	X_2: Level of Surfactant	X_3: Ultrasonication time	Y_1: Particle Size (nm)	Y_2: Entrapment Efficiency (%)
1	AP-NLCs1	Factorial	-1	-1	1	179.2	68.30±1.05
2	AP-NLCs2	Factorial	1	1	1	248.4	73.70±1.51
3	AP-NLCs3	Factorial	1	-1	-1	485.5	70.73±1.75
4	AP-NLCs4	Factorial	-1	-1	-1	424.1	58.10±1.57
5	AP-NLCs5	Factorial	1	-1	1	200.6	80.17±0.93
6	AP-NLCs6	Centre	0	0	0	289.5	77.30±1.67
7	AP-NLCs7	Factorial	1	1	-1	448.6	54.73±0.99
8	AP-NLCs8	Factorial	-1	1	1	276.2	42.27±1.46
9	AP-NLCs9	Centre	0	0	0	300.1	73.30±1.20
10	AP-NLCs10	Centre	0	0	0	309.8	82.23±1.19
11	AP-NLCs11	Factorial	-1	1	-1	405.4	40.57±1.42

Table 3: Composition of DoE trials of Prepared AP-NLCs

DoE Trials	Drug (mg)	Solid Lipid (Glyceryl Behenate in mg)	Liquid Lipid (Caprylocaproyl polyglycerides in mg)	Total Lipid Weight (mg)	Solid lipid/liquid lipid Ratio	Surfactant (Polysorbate 80 in mg)	Ultra-sonication time (min)
AP-NLCs1	125.0	90.0	210.0	300.0	3:7	24.0	20
AP-NLCs2	125.0	210.0	90.0	300.0	7:3	48.0	20
AP-NLCs3	125.0	210.0	90.0	300.0	7:3	24.0	10
AP-NLCs4	125.0	90.0	210.0	300.0	3:7	24.0	10
AP-NLCs5	125.0	210.0	90.0	300.0	7:3	24.0	20
AP-NLCs6	125.0	150.0	150.0	300.0	5:5	36.0	15
AP-NLCs7	125.0	210.0	90.0	300.0	7:3	48.0	10
AP-NLCs8	125.0	90.0	210.0	300.0	3:7	48.0	20
AP-NLCs9	125.0	150.0	150.0	300.0	5:5	36.0	15
AP-NLCs10	125.0	150.0	150.0	300.0	5:5	36.0	15
AP-NLCs11	125.0	90.0	210.0	300.0	3:7	48.0	10

Note: Amount of Purified water taken was 480 ml as aqueous phase

Statistical Analysis

The two factorial design was employed to study relationship between the formulation variables (independent variables: X_1, X_2, X_3) and dependent variables (Responses: Y_1, Y_2). The following polynomial equation was generated from software and experimental values are incorporated into it.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \varepsilon$$

Where Y represents the predicted response (Particle size, entrapment efficiency), β_0 is overall intercept, β_1 β_2 β_3 are individual intercept of main effect of X_1, X_2, X_3. The ANOVA studies are generated from the software to evaluate the p-values, F-ratio, and lack of fit. To evaluate the fitting extent of experimental data regression coefficient ($R^{2)}$ and adjusted R^2 were determined. 3D and contour plots prediction was done to run further experimental studies for estimation of model accuracy.

Characterization of AP-NLCs

Particle size distribution and Zeta potential

The particle size distribution and zeta potential of the nanoparticles was determined by laser particle size analyzer (Zetatrac 10.6.2, Microtrac Inc.) using distilled water as dispersant. The nanoparticle dispersions were added to the sample dispersion unit containing stirrer and stirred to reduce the aggregation between the nanoparticles. The average volume-mean particle size was measured after performing the experiment in triplicate.

Drug encapsulation efficiency (%)

Drug encapsulation efficiency (EE) was determined through indirect method where an aliquot (2 ml) of AP-loaded NLCs was centrifuged at 100,000 RPM for 2h at 4 ^{0}C using a Beckman Optima^{TM} Ultracentrifuge (Optima^{TM} XL, Indianapolis, IN). The proportion of unencapsulated AP in the clear supernatant fluid was measured spectrophotometrically (Shimadzu, the model UV-1800 PC, Kyoto, Japan) at 210 nm against blank. Calibration curve for the validated UV assay of AP was performed on six solutions in the concentration ranges of 2–20 µg/ml. Correlation coefficient was >0.999. Each point represents the mean of three measurements and standard deviation (±SD) was calculated. The encapsulation efficiency of AP-NLCs was then calculated according to the following equation:

EE% = (Da – Df / Da) * 100

Where, EE% is the percentage of encapsulation efficiency, Da is the amount of drug added during preparation of NLCs and Df is the amount of free drug in the clear supernatant fluid after centrifugation.

***In vitro* drug diffusion**

In vitro diffusion studies were carried out by using dialysis tubes with an artificial

membrane using an optimized formulation of AP-NLCs. The prepared Aprepitant nanoparticles were re-dispersed in 5 ml of recommended media *i.e.*, 2.2% SLS in purified water and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 50 ml of 2.2% SLS in purified water. The medium in the receptor was agitated continuously using a magnetic stirrer and the temperature was maintained at 37 ± 0.5 ^{o}C. 5 ml sample of receptor compartment was taken at various intervals of time over a period of 6h and each time replaced with 5 ml of fresh media. The amount of drug released was determined spectrophotometrically at 210 nm.

Differential scanning calorimetry (DSC)

To evaluate thermal behavior of a pure drug, DSC studies are applied. It is based on principle of measurement of heat flow in and out of sample and reference for the period of controlled temperature cycle, merely 5-10 mg sample was sealed in aluminum pan followed by heating at the rate the of 20 °C/min over the temperature range of 10-200 °C under liquid nitrogen flow rate 40 ml/min and thermogram was obtained.

Surface morphology study (SEM)

Scanning electron microscopy (Zeiss, TIFR, Mumbai) of an optimized formulation of AP-NLCs was performed to examine the particle size and surface morphology. The photographs were taken using a scanning electron microscope under magnification of 10 KX–12 KX. The nanoparticles were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument.

RESULTS

The purpose of this study was to develop lipid based nanoparticles of aprepitant for treatment of emesis after-treatment of chemotherapy. AP-NLCs were prepared by emulsification using high-speed homogenization followed by ultrasonication process. A 2^3 full factorial design with three centre point was employed.

Drug polymer interaction study

Fourier transform infrared spectroscopic (FTIR) analysis was employed to determine any interactions between drug and polymer. The FTIR studies revealed no chemical interaction between the drug and the polymer **(Table 4, Figure 1, 2)**.

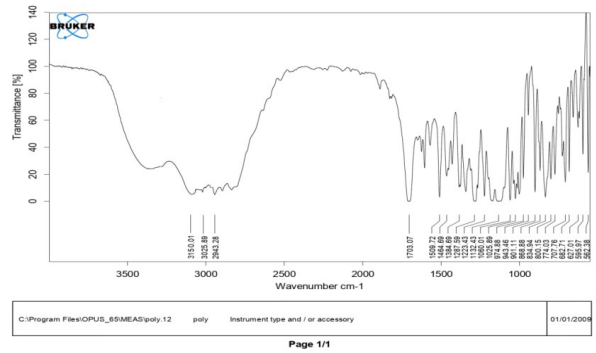

Figure 1: FTIR spectrum of Pure Drug Aprepitant

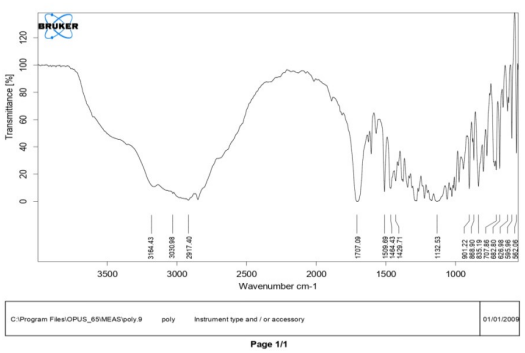

Figure 2: FTIR spectrum of Final Formulation

Table 4: FTIR table of functional groups of Aprepitant and its formulaton

Characteristic peak	Range	Drug (Aprepitant)	Formulation batch
Aliphatic C-H stretch	2850-3000	2943.28	2917.40
Aromatic C-H stretch	3050-3150	3025.89	3030.98
Amide Keto C=O stretch	1630-1880	1703.07	1707.09
N-H stretch	3100-3500	3150.01	3164.43
C-F stretch	1000-1400	1132.43	1132.53

Results of DSC (Figure 3)

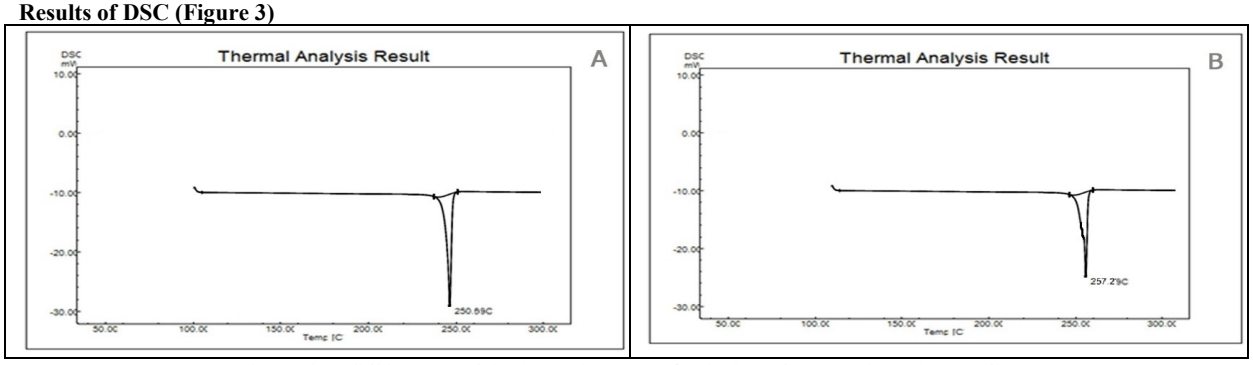

Figure 3: DSC images of A: Aprepitant, B: Optimized formulation (AP-NLCs)

SEM

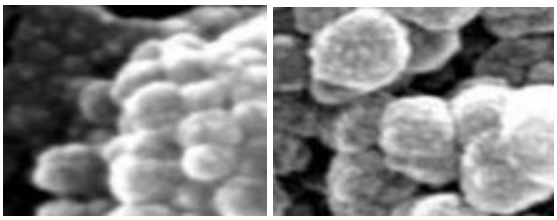

Figure 4: SEM images of optimized nanoparticulate formulation

Particle Size and Zeta Potential

The prepared AP-NLCs showed particle size between 179.2 and 485.5 nm while zeta potential values varied between 40.57 ± 1.05 ± and 82.23 ± 1.19 mV. The average diameter and zeta potential of optimized formulation was determined by photon correlation spectroscopy (PCS) at room temperature. The particle size of optimized formulation was found to be mean average of 200.6 nm with maximum intensity of 541.0 nm particles. As Zeta Potential is an important tool for prediction of long term stability and understanding the state of the nanoparticle surface. The value greater than +25 mV or less than –25 mV have high degree of stability. Zeta potential was found to be 40.57± 1.42 mV for optimized formulation, thus it suggests that the formed nanoparticles will be remain stable satisfactorily as shown in **Figure 5, 6**.

Entrapment Efficiency

Table 2 shows the percentage entrapment efficiency of formulations. The entrapment efficiencies of AP-NLCs ranged from 40.57 ± 1.42% to 82.23 ± 1.19%. The results of the present study demonstrated that the encapsulation efficiency of AP-NLCs was affected by the concentration of solid/liquid lipid proportion and surfactant concentration. The maximum percentage drug entrapment was obtained for the formulation AP-NLCs10. It was observed that increase in concentration of solid lipid and surfactant significantly increases percentage entrapment upto certain extent, but further increase in surfactant concentration from 7.5% to 10%, the percentage entrapment efficiency was reduced as observed for the formulation AP-NLCs11. however, the sonication time may not have impact on the percentage entrapment efficiency. The statistical equation generated from the DoE study was shown below for the entrapment efficiency response **(Figure 7)**.

$$Y_2 = +65.00 +14.25\,A+5.25B +2.75C +0.50AB - 0.50AC -0.50\,BC +1.25A$$

$$Y_1 = +313.13 +12.13A+11.13B-107.38C- 8.38AB-13.88AC+25.12BC-3.87ABC$$

***In-vitro* drug release studies**

In-vitro release studies were performed in 2.2% SLS in purified water. A slow release of Aprepitant up to 30hr was observed. DSC results indicated that the aprepitant entrapped in the nanoparticles existed in an amorphous or disordered-crystalline status in the polymer matrix. Scanning electron microscopy was done to study the surface morphology. Results revealed that more spherical shaped particles with rough surface were formed. The highest correlation

coefficients were obtained for the Higuchi model, suggesting a diffusion mechanism for the drug release. The results demonstrated that aprepitant nanoparticles with such ingredients could be an alternative delivery method for the long-term treatment of emesis.

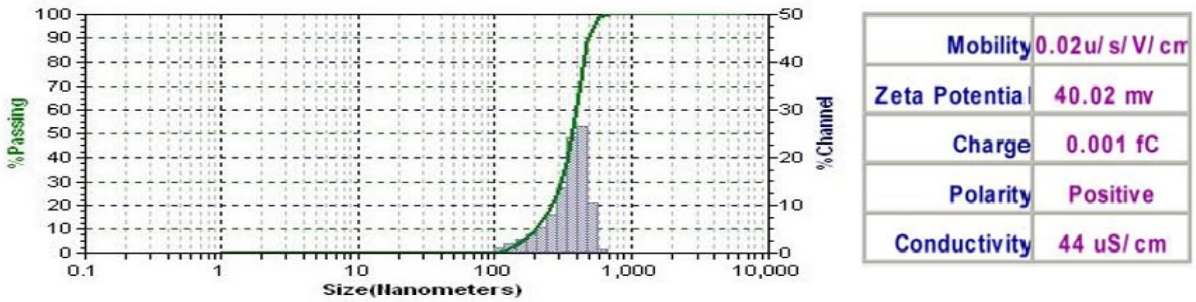

Figure 5: Particle size distribution and zeta potential of AP-NLCs

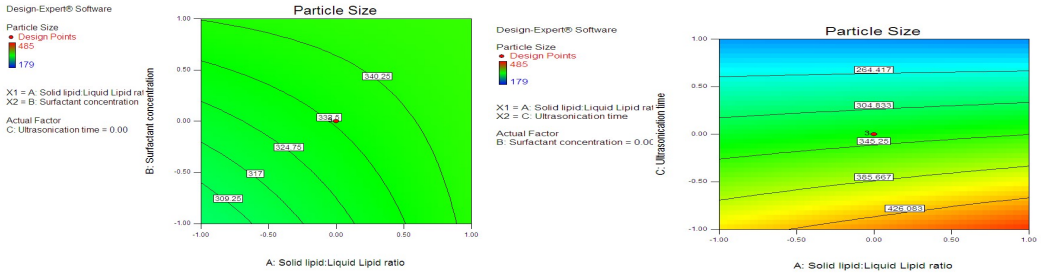

Figure 6: 2D images of contour plots with respect to particle size

BC

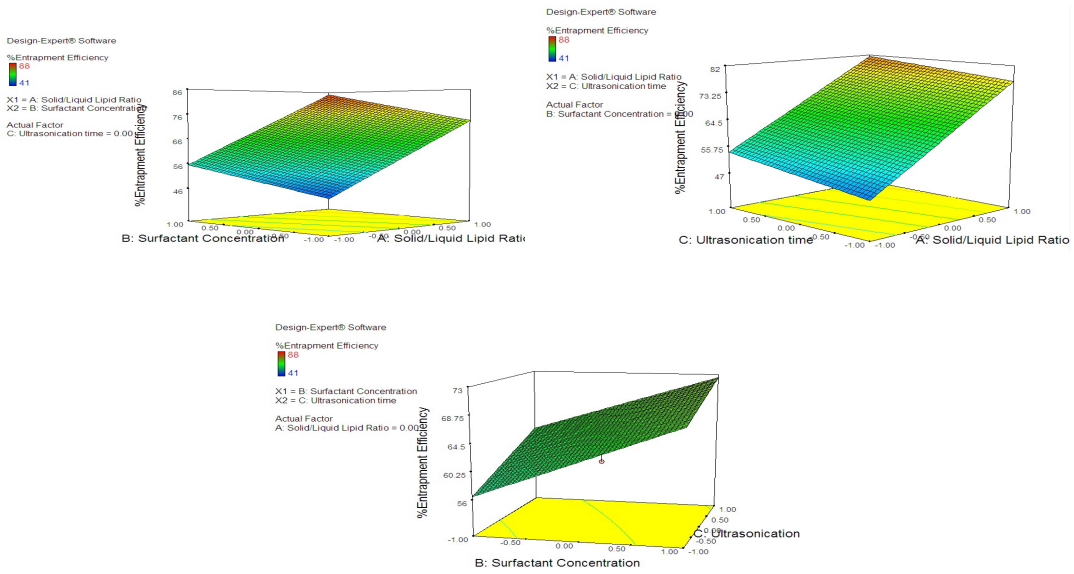

Figure 7: 3D images of contour plots with respect to %EE responses

Table 5: Design points of optimized formulation by experimental design

Variables	Coded Values (PS)	Coded values (EE)	Actual values (PS)	Actual values (EE)
X1	+1	+1	7:3 Ratio	7:3 ratio
X2	-1	-1	5.0 %	5.0 %
X3	+1	+1	20 min	20 min

Table 6: Statistics of Model

Statistical terms	Particle size (nm)	% Entrapment Efficiency
Standard Deviation	10.69	1.15
C.V %	2.38	1.80
R^2	0.9984	0.9986
Adj R^2	0.9928	0.9938
Adeq R^2	38.255	44.999

Table 7: Comparative Values of predicted and observed values of responses

Dependent variables	Predicted Response	Observed response	Predicted error %
Y_1	200.0	202.9	+1.429
Y_2	80.17	82.74	+1.123

DISCUSSION

Lipid based and polymeric nanocapsules/nanoparticles were developed for delivery of aprepitant. Lipidic materials such as stearic acid and glyceryl behenate were used to produce different types of carrier systems for aprepitant. All the formulations were extensively characterized and evaluated. Manufacturing conditions for the formulations were optimized using design of experiments approach.

The present research paper establishes a successfully optimized composition of nanaostructured lipid carriers of aprepitant, designed by QbD approach and optimized by DoE tool. The optimized composition was derived from the initial risk assessment, preformulation studies and using the design of experiment (DoE) concept. The initial risk assessment was carried out to identify the critical formulation variables (CFVs) and critical process parameters (CPPs) that have most likely impact on the critical quality attributes (CQAs). Based on the initial risk assessment, two CQAs, namely particle size and percentage entrapment efficiency were found to be most critical attributes for the AP-NLCs. In addition, the CQAs that are found to be of medium impact are Zeta potential and Dissolution rate. The CFVs found to be the solid lipid/liquid lipid ratio and surfactant concentration and the most critical CPP found to be the ultrasonication time. The impact of CFVs and CPP on the CQAs of the drug product were studied by the 2^3 full factorial design with three centre points. The optimized composition of AP-NLCs was found to be with solid to liquid lipid ratio of 7:3, with 5.0 % surfactant concentration and 20 minutes of

ultrasonication time i.e. AP-NLCs5 formulation.

The prepared NLCs showed particle size between 179.2 and 485.5 nm while zeta potential values varied between 40.02 ± 0.24 and 15.42 ± 0.31 mV. They also showed high encapsulation efficiency (>80%) and amorphous state of the drug in lipid matrix. The results demonstrated that aprepitant nanoparticles with Glyceryl behenate could be an alternative delivery method for the treatment of CINV. The difference between Predicted R^2 and Adjusted R^2 for both the responses are less than 0.2, as Adequate R^2 is more than 4 which is used to find the design space. The values of observed responses and predicted are less than 5% which is within the acceptable limit.

CONCLUSION

Based on these results, it can be concluded that, a promising Aprepitant loaded NLC could be developed which can be a potential alternate therapy for CINV. Future prospect of AP-NLCs would be facilitated by *in-vivo* performance with enhanced practical understanding.

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