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Research Article

Quality by Design Enabled Development and Optimization of the Nanoparticulate System of Cabazitaxel

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ABSTRACT

Cabazitaxel (CTX), a novel taxane derivative, has proven effective in many solid tumors. It is also approved in many countries for multiple uses in solid tumors. The current marketed formulation lacks the tumor-targeting ability, and its uneven distribution in the body causes toxicity to normal tissues. Further, it is a surfactant (polysorbate 80) based micellar formulation composed of ethanol as a co-solvent to improve the solubility of CTX, which causes severe and life-threatening side effects. Hence, to avoid the problem associated with this conventional CTX formulation, the nanoparticulate drug delivery system of CTX was developed by employing the Quality by Design (QbD) approach. The CTX nanoparticulate system was developed by employing a bottom-up followed by a top-down approach. The size reduction was obtained by High-Pressure Homogenizer (HPH). The formulation optimization was done using QbD approach. Design of experiments (DoE) was used to understand the effect of various formulation and process variables on a dependent variable like particle size distribution.

The stabilizer concentration, concentration of solubilizer, HPH pressure, and passes were selected as independent factors while particle size distribution was selected as a dependent factor for evaluation. The nanoparticulate system was developed using PEG-400 as solubilizing agents, while Soya Phosphatidylcholine (SPC) was used as a surface stabilizer. Response surface plots revealed a decrease in particle size with increasing concentration of SPC and PEG 400. Similarly, a decrease in particle size with increased HPH passes and pressure was found. The optimum concentrations of SPC and PEG 400 were found to be 20% and 2.5%, respectively. 20 KPSI pressure and 5 HPH passes were derived as optimized processing parameters from DoE. The optimized formulation had a size of 43.5 nm, with PDI < 0.4. Due to its narrow particle size distribution, the formulation did not show any increase in particle size or aggregation up to 24 hours. The present research confirms the feasibility of developing the nanoparticulate system of CTX using the bottom-up followed by the top-down technique. The formulation was systematically optimized using QbD approach. The optimum concentration of PEG 400 as solubilizer and concentration of SPC as stabilizer was obtained from DoE, yielding optimum particle size and stability.

Introduction

Nanoparticles are defined as colloidal dispersions or suspensions with a size in the range of around 100 nm. The drug candidate can be dissolved, entrapped, encapsulated, or attached to a nanoparticle matrix. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties, and release of pharmacologically active agents to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. The key advantages of using nanoparticles as a drug delivery system include a) passive drug targeting after parenteral administration can be achieved when particle size is less than 100 nm, b) the ability to modulate the release profile of the drug in sustain or controlled manner, c) drug degradation can be prevented by matrix constituents, and d) the system can

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be used for various routes of administration including oral, nasal, parenteral, and intra-ocular.[1] Despite the advances mentioned above, it is critical to design a nanoparticle preparation method that is eventually industrially feasible and scalable. Global regulatory bodies such as US Food & Drug Administration (US FDA) and European Medicines Agency (EMA) are mandated to assure the safety of inactive ingredients (excipients) used in the product. Therefore, the use of pharmaceutically acceptable and safe (non-toxic) excipients are almost warranted in designing nanoparticle drug delivery system.

QbD is a systematic, scientific, and risk-based proactive approach to pharmaceutical development, which comprises designing and developing formulations and manufacturing processes with predefined product specifications.[2] It is a systematic approach used to develop a product or process based on prior risk assessment of the variables that can directly impact the product or process.[3] The implication of QbD is strongly recommended by regulatory authorities such as US FDA and EMA. It can also help understand the important methods and product constraints that rely on risk measurement.[4] QbD encompasses the application of tools such as the design of experiments (DoE), risk assessment, and process analytical technology (PAT) for the development of pharmaceuticals.[5] DoE is an important tool of QbD, which allows understanding of the influence of formulation and process variables on the product quality by defining a 'design space' (DS).[6] DS provides the flexibility of operating within that space without further regulatory approvals

Cabazitaxel (CTX), a novel taxane derivative, effectively kills resistant cancer cells in vitro and preclinical animal models.[7,8] Many regulatory bodies of different countries have approved the market formulation of cabazitaxel (Jevtana, Sanofi-Aventis LLC, Bridgewater, NJ) as a new option for chemotherapy.[9] However, as with the case of most of the chemotherapeutic agents, the cabazitaxel suffers from poor physicochemical properties such as poor water solubility and dissolution. The current marketed formulation of cabazitaxel contains a surfactant (polysorbate 80) based micellar composition and ethanol as a co-solvent to improve the solubility of CTX.[10] However, polysorbate 80 as a solubility enhancer causes an increased risk of life-threatening hypersensitivity reactions, including generalized erythema, hypotension, and bronchospasm.[10] In addition, the current marketed formulation lacks the tumor-targeting ability, and its uneven distribution in the body causes toxicity to normal tissues. Hence, alternate formulation strategies need to be explored to utilize the therapeutic potential of this drug to its fullest extent.

Researchers have made various attempts to resolve the poor water solubility of CTX. Markus Fusser *et al.* reported poly (2-ethyl-butyl cyanoacrylate) (PEBCA) nanoparticles of cabazitaxel for overcoming solubility issues and improving treatment efficacy in a patient-derived breast cancer xenograft. Despite good therapeutic efficacy, prepared nanoparticles formulation had suffered from several limitations such as multistep and lengthy preparation process (sonication, polymerization, and dialysis), low feasibility of commercial scale-up due to the complexity of the process, and most important is uncertainty on the long-term safety of poly (2-ethyl-butyl cyanoacrylate) polymer.[11] Similarly, Gdowski *et al.* reported poly(DHL-lactic-co-glycolic acid) nanoparticles of CTX for improved drug delivery to the bone microenvironment [12]. However, the method involved using chloroform and ethyl acetate as toxic and unacceptable solvents.

Considering the limitations of the existing marketed formulation (Jevtana) and the reported studies, the present study aimed to develop a nanoparticles system of CTX to overcome the problem of poor solubility and toxicity associated with polysorbate surfactant. The developed nanoparticulate system will aid the solubility of the drug and enhance the delivery of drugs to tumor sites by passive targeting, owing to its nano-size. The dispersion media can be water, aqueous solutions, or non-aqueous media (e.g., liquid polyethylene glycol [PEG]). [13] To develop the cabazitaxel nanoparticulate system, biocompatible lipids such as phospholipid would be used. This amphiphilic nature of phospholipids makes them a most suitable choice as excipients for poorly water-soluble drugs as they serve the role of both stabilizer and carrier.[14] Additionally, in the present work, a quality by design (QbD) based approach was used to optimize the formulation and processing parameters. The number of homogenization passes and homogenization pressure was identified as critical process parameters (CPP), whereas the concentration of stabilizer and solubilizer were identified as critical material attributes (CMA). Particle size distribution and polydispersity index were chosen as critical quality attributes (CQA) to get desired quality target product profile (QTTP). Overall, the current study reports QbD based systematic approach in developing a nanoparticles system of CTX to overcome the problems of poor solubility and toxicity associated use of existing formulations. Thus present study offers mainly two advantages: 1) implementation of QbD approach in designing the nanoparticulate system, and 2) Usage of safe, generally accepted by regulatory bodies and novel excipients to design the nanoparticulate drug delivery system.

Materials and Method

Materials

Cabazitaxel (assay~100.2% w/w) was generously provided by Intas Laboratories Ltd. (Ahmedabad, India). Soy phosphatidylcholine, C18:2 (SPC), was purchased

from Lipoid (Ludwigshafen, Germany). Monobasic citrate anhydrous and sucrose low endotoxin levels were purchased from Merck Specialities Pvt. Ltd (Mumbai, India). All other chemicals and reagents were of analytical grade and used without further purification. Purified Milli-Q water (Millipore, Billerica, MA, USA), degassed and filtered through 0.45 µm hydrophilic PVDF filter (Millipore Millex-HV) was used in all experiments.

Analytical Method Development for CTX Estimation

High-Performance Chromatography (HPLC) method was used to estimate CTX in all samples, including the solubility and dissolution experiments. The HPLC system comprised a Shimadzu with Chromeleon software with a quaternary pump, an autosampler unit, and an LC-2010C HT with UV detector. YMC Pack Pro C18 RS 3µ, (150 mm × 4.6 mm) (YMC Co. Ltd) analytical column was used to estimate. The mobile phase consisted of water and acetonitrile in 30:70 (% v/v) proportions. The flow rate was maintained at 1.2 mL/min, the injection volume is 20 µL, and the UV detector was set at 232 nm. The Chromeleon software was used for the analysis of results. The method was validated as per the ICH guideline.

Experimental Design

The CTX nanoparticulate system was generated by a bottom-up and top-down technique combination. Briefly, 2 mg/mL of CTX was dissolved in an aqueous solution containing 3.0% w/v PEG 400 at 55°C under continuous stirring at 400 RPM for 15 minutes. Meanwhile, 40 mg/mL of soy phosphatidylcholine was dispersed in sodium citrate buffer using a high shear homogenizer (Ultra-Turrax T25, IKA, India) at 4000 RPM for 15 minutes. The resultant suspension was subjected to size reduction using a high-pressure homogenizer (EmulsiFlex-C3, Avestin, Inc., Canada) initially at 10 kpsi (1 cycle) and then at 25 kpsi (2 cycles). Mix CTX solution to lipid suspension at 25°C under high shear homogenizer (Ultra-Turrax T25, IKA, India) and then pass through high-pressure homogenizer (EmulsiFlex-C3, Avestin, Inc., Canada) at 10 to 15 kpsi (2 cycles). The sample was stored at 2-8°C for further use

Initial screening studies evaluated the effect of formulation parameters on CTX nanoparticulate system formulation and its stability. The number of homogenization passes and pressure was identified as critical process parameters (CPP), whereas soy phosphatidylcholine concentration (mg/mL) and polyethylene glycol 400 (%w/v) were identified as critical material attributes (CMA). The DoE was employed systematically to evaluate and optimize the selected formulation parameters (CPP and CMA) at three levels (-1, 0, +1). Based on the number of factors and their levels, a rotatable central composite design (CCD) was selected to investigate their effects on the nanoparticulate system's critical quality attributes (CQAs). The concentration of CTX (2.0 mg/mL) and monosodium citrate anhydrous (2.0 mg/mL) were kept constant in the experimental trials. Independent factors and their levels used in this study are shown in Table 1 while details about responses are given in Table 2. The design contains 30 experimental runs; i.e., sixteen (24) factorial points, eight (2×4) axial points, and six center points were generated and analyzed by the statistical software package Design-Expert® 13.0 (Stat-Ease Inc., USA).

Statistical Analysis

The statistical analysis of optimization batches was performed by Design Expert® Ver.12 (Stat-Ease Inc., Minneapolis, MN 55413) software. All statistical analyses regarding DOE batches were performed using the same software. Response surface plots overlaid contour plots were generated using the same software.

Evaluation of Nanoparticulate System of CTX

Drug and Lipid Quantification

Drug quantification was done as per the method described in the above section.

Lipid quantification was performed using HPLC with UV Detector. Soy phosphatidylcholine was quantified using a YMC Pack Pro C18 RS (250 x 4.6 mm), 5µ (Make: YMC Co. Ltd), or equivalent column with a UV detector. The mobile phase consists of acetonitrile/Methanol (80:20). Methanol

Table 1: Formulation variables and their levels for DoE

Independent factors (CPP/CMA)			*Design level*	
Actual parameters	*Unit*	*Coded*	*Actual value*	*Coded level*
Soy phosphatidylcholine concentration	mg/mL	A	10.0 20.0 30.0	-1 0 +1
PEG 400	% w/v	B	1.5 2.5 3.5	-1 0 +1
Number of HPH pass	No.	C	3 5 7	-1 0 +1
Pressure during pass	kpsi	D	15 20 20	-1 0 +1

Table 2: Studied responses and their constraints

Responses (Dependent variables/CQA)	*Constraints (Goal/QTTP)*
Mean Particle size distribution 0 hour (nm)	NMT 100 nm
Mean Particle size distribution 8 hours (nm)	NMT 100 nm
Mean Particle size distribution 24 hours (nm)	NMT 100 nm
Polydispersity index 0 hour (nm)	NMT 0.5
Polydispersity index 8 hours (nm)	NMT 0.5
Polydispersity index 24 hours (nm)	NMT 0.5

was used as a diluent to prepare samples.

Particle Size Measurement

Particle size distribution was measured using the Dynamic light scattering technique (Nano ZS, Malvern instrument). Analysis was performed by diluting the sample 10 times with water and measuring at a 173° angle.

Drug Degradation

Method for the related compounds (drug degradation) of CTX was developed at the laboratory by HPLC methods. The analytical column was YMC Pack Pro C18 RS (250 x 4.6 mm), 5µ (Make: YMC Co. Ltd) or equivalent, with a column temperature of 60 °C. The mobile phase was composed of acetonitrile: methanol (80:20). The detection wavelength was 232 nm and a 1.2 mL/min flow rate.

%Drug Association

%drug association was measured by subtracting the free CTX from the total CTX. Free CTX content in the nanoparticulate system was measured after separation. Free CTX was separated using a solid-phase extraction cartridge (Oasis® HLB 1cc (30 mg) extraction cartridge). The quantification of the separated drug was measured as described in the previous section. Associated CTX was calculated using the formula:

%Associated CTX=((%content of total CTX-Content of % free CTX)*100)/(%content of total CTX)

Stability Study

The optimized formulation of CTX nanoparticles was filled in a type 1 glass vial, capped with a rubber stopper, lyophilized, and kept at 25°C for stability study for three months. Various stability tests like appearance, an assay of drug and lipid, drug degradation, %drug association, and particle size were measured at different time points.

Results

Effect of Factors on the Responses

Effect of SPC concentration (A), PEG 400 concentration (B), Number of HPH passes (C), and Pressure during the pass (D) on various measured responses was summarised in Table 3.

Evaluation of Dependent Variables

Response: 1 Effect of Independent Variables on PSD at 8 hours

The selected factors were statistically analyzed and the results of ANOVA analysis are represented in Table 4.

The Model F-value of 5.15 implies the model is significant. There is only a 0.39% chance that this large F-value could occur due to noise.

p-values less than 0.0500 indicate model terms are significant. In this case C, D are significant model terms. Values greater than 0.1000 indicate that the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 0.40 implies that the Lack of Fit is insignificant relative to the pure error. There is a 93.86% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good. We want the model to fit.

The Predicted R^2 of 0.1707 is not as close to the Adjusted R^2 of 0.3721 as one might normally expect, i.e., the difference is more than 0.2. This may indicate a large

Table 3: Matrix of experiments of central composite design and measured responses

	Independent variables				*Dependent variables*			
Run#	*A*	*B*	*C*	*D*	*PSD 8 hours (nm)*	*PSD 24 hours (nm)*	*PDI 8 hours (nm)*	*PDI 24 hours (nm)*
1	20.00	2.5	5	20	42.9	43.1	0.356	0.338
2	10.00	1.5	7	25	29.5	29.8	0.576	0.542
3	30.00	3.5	3	15	44.7	46.7	0.516	0.503
4	20.00	2.5	5	20	47.9	46.4	0.382	0.372
5	30.00	1.5	7	25	28.7	27.0	0.314	0.317
6	10.00	1.5	3	15	73.6	56.7	0.288	0.389
7	40.00	2.5	5	20	37.7	33.5	0.331	0.361
8	30.00	1.5	3	25	38.1	34.5	0.359	0.364
9	20.00	2.5	5	20	39.4	36.3	0.352	0.371
10	20.00	0.5	5	20	48.7	46.3	0.308	0.305
11	10.00	3.5	7	15	36.8	42.7	0.254	0.32
12	10.00	1.5	7	15	58.5	54.1	0.286	0.297
13	30.00	3.5	3	25	38.8	35.9	0.366	0.419
14	20.00	2.5	5	10	35.6	35.9	0.331	0.316
15	10.00	3.5	7	25	28.1	28.3	0.358	0.342
16	20.00	2.5	1	20	103.1	163.2	0.202	0.373
17	20.00	2.5	9	20	34.7	32.8	0.343	0.331
18	20.00	2.5	5	20	43.9	42.9	0.320	0.307
19	20.00	2.5	5	20	42.1	40.8	0.314	0.298
20	10.00	1.5	3	25	32.2	30.1	0.426	0.429
21	20.00	2.5	5	10	104.7	105.3	0.174	0.368
22	20.00	2.5	5	20	47.0	44.9	0.324	0.309
23	30.00	1.5	3	15	58.4	61.2	0.325	0.433
24	30.00	3.5	7	15	45.9	47.0	0.392	0.359
25	10.00	3.5	3	25	31.4	30.8	0.491	0.490
26	30.00	1.5	7	15	40.1	35.3	0.334	0.345
27	0.00	2.5	5	20	1112.6	6987.2	0.284	0.369
28	20.00	4.5	5	20	38.8	38.9	0.349	0.315
29	10.00	3.5	3	15	52.8	55.4	0.359	0.335
30	30.00	3.5	7	25	33.2	31.9	0.339	0.408

block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Your ratio of 7.527 indicates an adequate signal. This model can be used to navigate the design space.

The response plots, including contour plots and 3D surface plots of all the significant model terms are depicted in the Figs. 1 and 2.

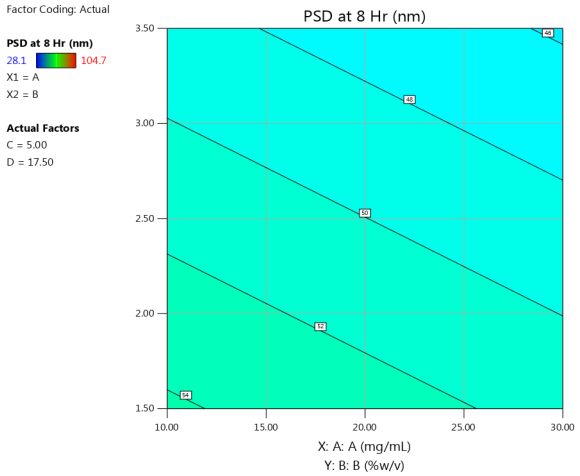

Fig. 1: Contour plot for the effect of the independent variable on PSD at 8 hours

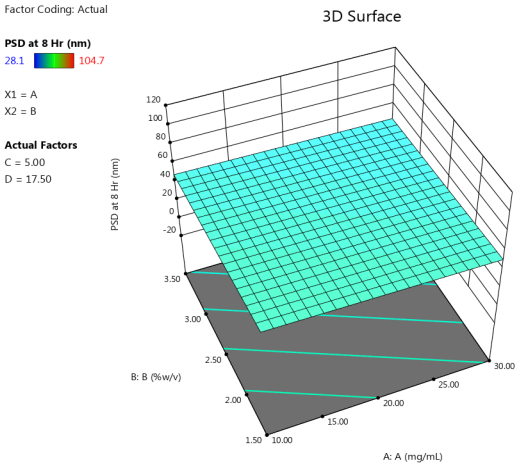

Fig. 2: 3D surface plot for the effect of the independent variable on lipid degradation

The result of PSD at 8 hr was independent of SPC concentration and PEG 400 concentration in the studied range.

Response: 2 Effect of Independent variables on PSD at 24 hours

The selected factors were statistically analyzed and the results of ANOVA analysis are represented in Table 5.

The Model F-value of 3.03 implies the model is significant. There is only a 3.74% chance that an F-value this large could occur due to noise.

Table 4: ANOVA analysis of response: PSD at 8 hours

Source	*Sum of Squares*	*df*	*Mean Square*	*F-value*	*p-value*	
Model	4599.14	4	1149.79	5.15	0.0039	significant
A-A	42.12	1	42.12	0.1886	0.6680	
B-B	188.16	1	188.16	0.8424	0.3678	
C-C	1768.17	1	1768.17	7.92	0.0096	
D-D	2591.81	1	2591.81	11.60	0.0023	
Residual	5360.65	24	223.36			
Lack of Fit	2923.15	18	162.40	0.3997	0.9386	not significant
Pure Error	2437.50	6	406.25			
Cor Total	9959.79	28				

Table 5: ANOVA analysis of response: PSD at 24 hours

Source	*Sum of Squares*	*df*	*Mean Square*	*F-value*	*p-value*	
Model	6817.70	4	1704.42	3.03	0.0374	significant
A-A	52.02	1	52.02	0.0924	0.7638	
B-B	25.63	1	25.63	0.0455	0.8329	
C-C	4160.67	1	4160.67	7.39	0.0120	
D-D	2569.61	1	2569.61	4.56	0.0431	
Residual	13518.81	24	563.28			
Lack of Fit	11047.87	18	613.77	1.49	0.3256	not significant
Pure Error	2470.94	6	411.82			
Cor Total	20336.50	28				

P-values less than 0.0500 indicate model terms are significant. In this case C, D are significant model terms. Values greater than 0.1000 indicate that the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 1.49 implies that the Lack of Fit is insignificant relative to the pure error. There is a 32.56% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good; we want the model to fit.

A negative Predicted R^2 implies that the overall mean may better predict your response than the current model. In some cases, a higher-order model may also predict better.

Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Your ratio of 5.344 indicates an adequate signal. This model can be used to navigate the design space.

The response plots, including contour plots and 3D surface plots of all the significant model terms are depicted in the Figs. 3 and 4.

The results of PSD at 24 hr were independent of SPC concentration and PEG 400 concentration.

Response: 3 Effect of Independent variables on PDI at 8 hours

The selected factors were statistically analyzed and the results of ANOVA analysis are represented in Table 6.

The Model F-value of 3.19 implies the model is significant. There is only a 1.57% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case, D and AD are significant

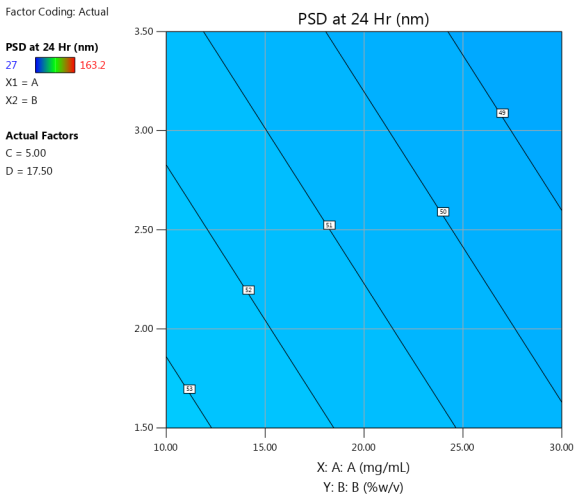

Fig. 3: Contour plot for the effect of the independent variable on PSD at 24 hours

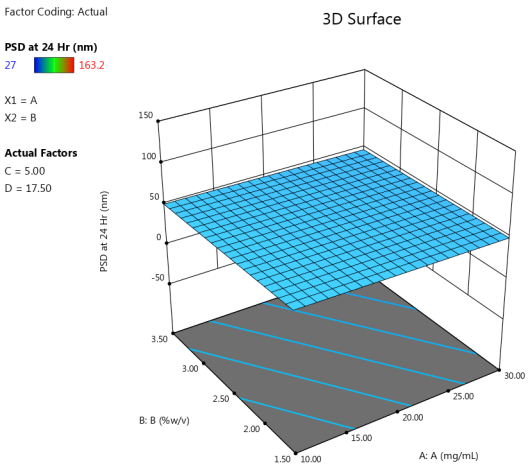

Fig. 4: 3D surface plot for the effect of the independent variable on PSD at 24 hours

Table 6: ANOVA analysis of response: PDI at 8 hours

Source	*Sum of Squares*	*df*	*Mean Square*	*F-value*	*p-value*	
Model	0.1183	10	0.0118	3.19	0.0157	significant
A-A	0.0060	1	0.0060	1.61	0.2211	
B-B	0.0094	1	0.0094	2.52	0.1297	
C-C	0.0004	1	0.0004	0.1180	0.7351	
D-D	0.0312	1	0.0312	8.41	0.0095	
AB	0.0098	1	0.0098	2.63	0.1224	
AC	0.0006	1	0.0006	0.1584	0.6953	
AD	0.0455	1	0.0455	12.25	0.0026	
BC	0.0157	1	0.0157	4.23	0.0546	
BD	0.0105	1	0.0105	2.82	0.1106	
CD	0.0017	1	0.0017	0.4696	0.5019	
Residual	0.0668	18	0.0037			
Lack of Fit	0.0510	12	0.0043	1.61	0.2885	not significant
Pure Error	0.0158	6	0.0026			
Cor Total	0.1851	28				

model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 1.61 implies that the Lack of Fit is insignificant relative to the pure error. There is a 28.85% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

A negative Predicted R^2 implies that the overall mean may better predict your response than the current model. In some cases, a higher-order model may also predict better.

Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Your ratio of 8.152 indicates an adequate signal. This model can be used to navigate the design space.

The response plots, including contour plots and 3D surface plots of all the significant model terms are depicted in the Figs. 5 and 6.

The result of PDI at 8 hours was found to be directly proportional to SPC concentration and PEG 400 concentration.

Response: 4 Effect of Independent variables on PDI at 24 hours

The selected factors were statistically analyzed, and the results of ANOVA analysis are represented in Table 7.

The Model F-value of 3.09 implies the model is significant. There is only a 2.37% chance that this large F-value could occur due to noise.

p-values less than 0.0500 indicate model terms are significant. In this case, AD is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 2.97 implies a 9.23% chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad -- we want the model to fit. This relatively low probability (<10%) is troubling.

A negative Predicted R^2 implies that the overall mean may better predict your response than the current model.

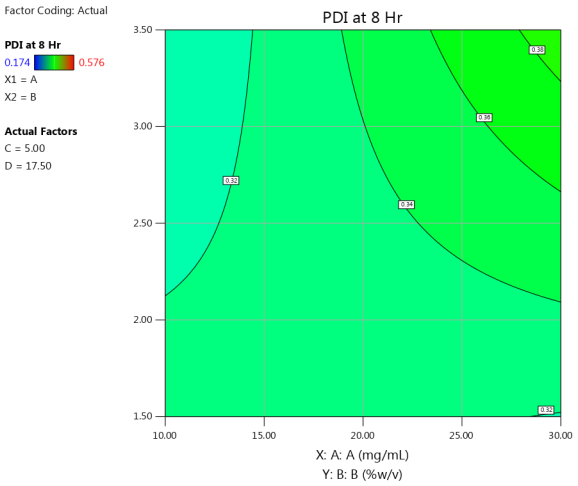

Fig. 5: Contour plot for the effect of the independent variable on PDI at 8 hours

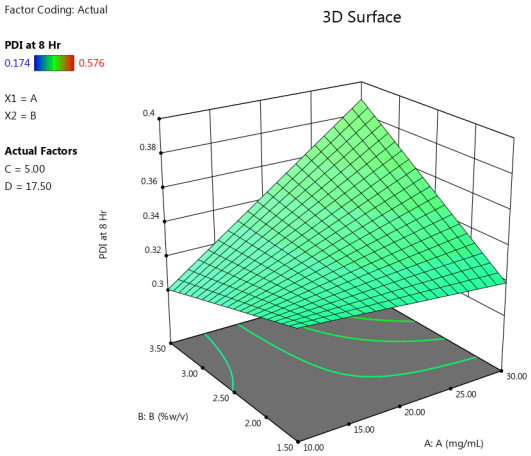

Fig. 6: 3D surface plot for the effect of the independent variable on PDI at 8 hours

Table 7: ANOVA analysis of response: PDI at 24 hours

Source	*Sum of Squares*	*df*	*Mean Square*	*F-value*	*p-value*	
Model	0.0514	6	0.0086	3.09	0.0237	significant
A-A	0.0048	1	0.0048	1.75	0.1999	
B-B	0.0003	1	0.0003	0.0964	0.7592	
C-C	0.0111	1	0.0111	4.01	0.0577	
D-D	0.0080	1	0.0080	2.88	0.1040	
AB	0.0100	1	0.0100	3.61	0.0705	
AD	0.0221	1	0.0221	7.97	0.0099	
Residual	0.0609	22	0.0028			
Lack of Fit	0.0541	16	0.0034	2.97	0.0923	not significant
Pure Error	0.0068	6	0.0011			
Cor Total	0.1123	28				

In some cases, a higher-order model may also predict better.

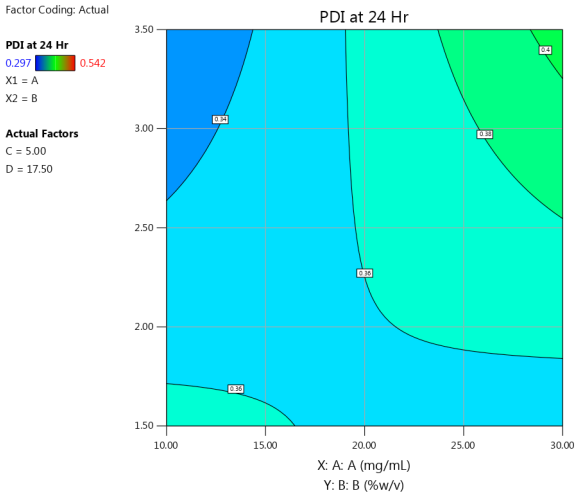

Fig. 7: Contour plot for the effect of the independent variable on PDI at 24 hours

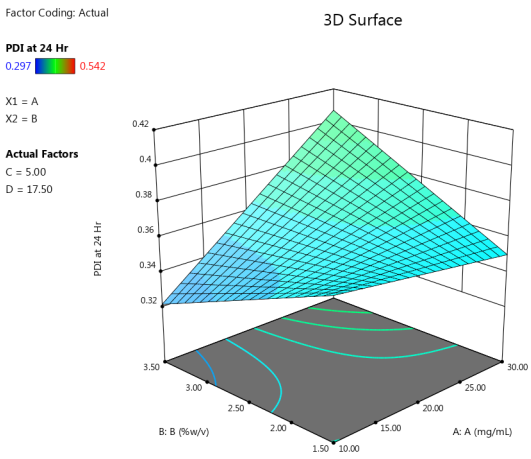

Fig. 8: 3D surface plot for the effect of the independent variable on PDI at 24 hours

Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Your ratio of 7.639 indicates an adequate signal. This model can be used to navigate the design space.

The response plots, including contour plots and 3D surface plots of all the significant model terms are depicted in the Figs. 7 and 8.

The result of PDI at 24 hr was found to be directly proportional to SPC concentration and PEG 400 concentration.

Checkpoint Batches and Cross-validation of DOE Model

Two experiments were performed at different parameters of SPC concentration (A), PEG 400 concentration (B), Number of HPH passes (C), and Pressure (D) to check the reliability of the model at values other than those used in experimental design. Bias or % relative error was calculated for each response as per the following equation;

$$\% \text{ Bias} = [(\text{Predicted Value} - \text{Experimental value}) / (\text{predicted value}) \times 100\%] \quad (5)$$

From the data, it can be deduced that the equations satisfactorily demonstrate the influence of process variables on the responses of the study due to a fairly good agreement between the predicted and experimental values in both checkpoint batches.

Stability Study

Stability study of the optimum batch was performed at 2–8 °C and 25°C storage conditions for up to six months. The vials were withdrawn and tested for appearance, an assay of drug and lipid, drug degradation, %drug association, and particle size as per the stability plan. Stability result at 2–8°C and 25°C conditions was satisfactory for up to six months. The summary of results is presented in Table 8.

Discussion

Biopharmaceutics classification system (BCS) class II and IV drugs pose a significant challenge of low

Table 8: Stability data of optimized formulation of CTX nanoparticulate system

		Storage condition				
			2–8°C		*25°/60%*	
Parameters	*Limits*	*Initial*	*3M*	*6M*	*3M*	*6M*
Assay of Cabazitaxel (%)	90%-110%	100.8	100.5	99.5	99.4	99.9
SPC Content, %	70%-130%	108.8	108.8	107.3	108.6	107.9
Total Impurities	NMT 3.0%	0.251%	0.293	0.261	0.286	0.395
%Drug association	More than 90%	92.1	98.70	95.00	96.90	94.00
Particle Size Distribution, nm	Mean Dia. (Less than 200 nm)	33.2	35.6	37.3	42.2	37.3
	D10	15.1	17.1	18.1	21.4	17.8
	D50	29	31.6	33.3	38.1	33.1
	D90	57	58.9	61.5	68.0	62.1
	D99	99.1	97.9	101.5	109.1	103.7

solubility.[15] Numerous approaches have been adopted to increase the solubility of these molecules, including the use of co-solvents,[16] surfactants,[17,18] lipid-based formulations,[19] inclusion complexation with cyclodextrin,[20] amorphous solid dispersions,[21] and nanotechnology.[22] Cabazitaxel is one such BCS class IV drug with limited solubility and permeability. It is supplied as a micellar solution for intravenous infusion by the name Jevtana®, composed of a surfactant (polysorbate 80) and ethanol as a co-solvent, to improve the solubility of CTX.[23] However, this marketed formulation is associated with serious adverse effects like neutropenia, thrombocytopenia, anemia, renal failure, and life-threatening hypersensitivity reactions associated with polysorbate 80. A CTX nanoparticulate system, free from polysorbate 80 was developed to obviate these limitations and further improve the tumor targeting of the drug. The advantages of nanoparticles such as a) avoidance of harsh vehicles (pH extremes, surfactants, or organic solvents, b) Passive targeting to tumours due to its nano-size, c) Large dose in small volume make them an ideal delivery system for anticancer drugs.

The CTX loaded nanoformulation was developed systematically using the QbD approach. The selection of excipients like surfactants, surface stabilizers, solubilizing agents were screened using Design of Experiment (DoE). Various formulations using these excipients were studied and the effect of these excipients on particle size and stability of the nanoparticulate system was assessed. The CTX, SPC, and polyethylene glycol 400 (PEG 400) were critical formulation components. Bottom-up followed by top-down technology was adopted to develop the CTX loaded nanoparticulate system. SPC was used as a surface stabilizer. Adsorption of SPC onto the drug surface, facilitated by the interaction of a hydrophobic group of SPC with drug molecule provides better surface coverage, thus stabilizing the CTX nanoparticulate system. Further reduction in the particle size of micro precipitated dispersion was achieved by the cavitation energy and attrition mechanism when the dispersion was subjected to a high-pressure homogenization process.

The formulation was systematically optimized by using Central Composite Design by taking SPC concentration, PEG 400 concentration, and HPH pressure & number of HPH passes as independent variables. Particle size and PDI were identified as dependent variables.

In the response surface plots, it was observed that the increase in SPC concentration gradually decreases the CTX nanosuspension particle size. Similarly, the DOE study showed CTX nanosuspension particle size decreases with the increasing concentration of PEG 400, which could be attributed to changes of API precipitation at higher PEG 400 concentrations. Due to Ostwald ripening, a narrow particle size distribution is essential to prevent particle growth. In all the studied formulations, the optimum formulation was found to be a size of 43.5 nm, with PDI < 0.4 and stability up to 24 hours. The optimized formulation was obtained at an SPC concentration of 20%, 2.5% concentration of PEG 400, and 5 HPH passes of 20 KPSI pressure. In this study, using the design of the experimental approach, we optimized all processing parameters and formulation variables to get desired properties like desired particle size, and optimized SPC content and PEG 400 content.

Abbreviations

CTX: Cabazitaxel, HPH: High-pressure homogenization, HPLC: High-Performance Chromatography, mg: mili gram, RP-HPLC: Reverse Phase High-Performance Liquid Chromatography, DoE: design of experiment

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