Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

In-vitro Evaluation and Characterization of the Nanoparticulate System of Novel Taxane Derivative

Mahesh Paithankar[1]*, Mangesh Bhalekar[2]

[1]*Intas Pharmaceuticals Ltd, Ahmedabad, Gujarat, India*
[2]*Department of Pharmaceutics, AISSMS College of Pharmacy, Pune, Maharashtra, India*

ARTICLE INFO

Article history:
Received: 04 March, 2022
Revised: 17 March, 2022
Accepted: 21 March, 2022
Published: 30 March, 2022

Keywords:
Cabazitaxel,
Design of experiment,
Nanoparticulate system,
Optimization,
Top-down.

DOI:
10.25004/IJPSDR.2022.140218

ABSTRACT

Cabazitaxel (CTX) is a novel taxane derivative indicated in the treatment of docetaxel-resistant metastatic prostate cancer. However, as with the case of most of the chemotherapeutic agents, CTX suffers from poor physicochemical properties such as low water solubility and dissolution. Current marketed formulation of CTX (sold under brand name 'JEVTANA' by Sanofi-Aventis) contains toxic surfactant, polysorbate 80 and organic solvent ethanol to improve its solubility. However, the use of polysorbate 80 as a solubility enhancer causes increased the risk of life threatening hypersensitivity reactions, generalized erythema, hypotension, and bronchospasm. Hence, to avoid the problem associated with this conventional CTX formulation, the nanoparticulate drug delivery system of CTX was developed by employing the QbD approach. Previous study included use of QbD approach to design and optimise nano particulate system of CTX. Multidisciplinary aspects of nanoscience and nanotechnology require broad range of characterization. In fact, quite often a wider characterization of NPs gives idea about its *in vivo* behaviour as well as its in use stability. The present work emphasizes a "Molecule Centric Approach", wherein formed nanoparticulate system was evaluated for quantification of drug component, particle size, surface potential, solid state characterization, *in vitro* drug release study, compatibility study, Photostability study, thermal cycling study reconstitution stability and dilution stability study. Formulation has been characterized and evaluated concerning their performance in in-vivo and their end patient usage. Our in-vitro characterization of nanoparticle formulation provides an indirect indication of good robustness, market acceptance and regulatory acceptance.

Introduction

Nanoparticles (NPs) has provided a promising novel platform for modulating physicochemical and biopharmaceutical properties and serve as a potential alternative to the aforementioned traditional approaches. NPs are defined as colloidal dispersions or suspensions with a particle size around 100 nm. The drug candidate can be dissolved, entrapped, encapsulated, or attached to a nanoparticulate system.[1] One of the key objectives of designing NPs as a delivery system is to improve biopharmaceutical properties of poorly water soluble drug molecules. The key advantages of using NPs 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, d) better drug tolerability and hence potentially improved efficacy due to increased dose, and e) the nanoparticulate system can be used for different routes of administrations (such as oral, nasal, parenteral, and intra-ocular).[2,3] Despite aforementioned advances, it is very critical and challenging to design a nanoparticle preparation method that is eventually feasible at industrial/large scale. Global regulatory bodies such as US Food and Drug Administration (US FDA) and European Medicines Agency (EMA) are mandating to assure the

***Corresponding Author:** Mahesh Paithankar

Address: Intas Pharmaceuticals Ltd, Ahmedabad, Gujarat, India

Email ✉: mpaithankar@intaspharma.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

safety of inactive ingredients (excipients) used in the product.[4] Therefore, use of pharmaceutically acceptable and safe (non-toxic) excipients are almost warranted in designing nanoparticle drug delivery system.

Recently, the development of drug delivery which delivers controlled drug release at the tumor sites emerged as an attractive option for enhancing anticancer therapeutics. Next-generation Nanotherapeutics must not contain the only nanoscale but should find their way to the solid tumor via active or passive targeting. However, successful translation of such complex formulations into the clinic relies on understanding critical physicochemical characteristics.

These include quantification of drug component, particle size, surface potential, solid state characterization, in vitro drug release study, compatibility study, Photostability study and thermal cycling study. They determine the pharmacokinetics of the formulation as well as robustness and suitability of the formulation for end patient use. Aim of this work is to characterize and evaluate cabazitaxel nanoparticulate system. In this research we have focused basically on the characterization of some parameters which are going to play an important role in in vivo performance and in-use stability of formulation.

Various attempts have been made by researchers to resolve the issue of poor water solubility of CTX. Markus Fusser *et al.,* fabricated poly (2-ethyl-butyl cyanoacrylate) (PEBCA) NPs of cabazitaxel to improve solubility issues and efficacy in a patient-derived breast cancer xenograft. Despite good therapeutic efficacy, work suffered by several limitations such as lack of in use and finished product stability data, lacking in in-vitro characterization.[5] Similarly, Gdowski *et al.* formulated poly (DHL-lactic-co-glycolic acid) NPs of CTX for improved targeted drug delivery to the bone microenvironment.[6] This work is also lacking in the extensive characterization with respect to in-use stability studies.

Considering the limitations of the existing literature and availability of data that make formulation robust, the present study aimed to characterize in vitro extensively that gives idea about in-use stability of the product. The present study emphasizes a "robustness of formulation", wherein formed nanoparticulate system was evaluated for quantification of drug component, particle size, surface potential, solid state characterization, *in vitro* drug release study, compatibility study, photostability study, thermal cycling study reconstitution stability and dilution stability study. Formulation has been characterized and evaluated concerning their performance in in-vivo and their end patient usage. Our *in-vitro* characterization of nanoparticle formulation provides an indirect indication of good robustness, market acceptance and regulatory acceptance.

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 for CTX Quantification

The high performance liquid chromatography (HPLC) method was used for the quantification of CTX in all samples, including assay and in vitro release experiments. The method was adopted from literature with slight modifications as per the requirements.[7] The HPLC system was a Shimadzu LC-2010C HPLC system with Chromeleon software equipped with a quaternary pump, an auto sampler unit, and UV detector. YMC Pack Pro C18 RS 3µ, (150 mm × 4.6 mm) (YMC Co. Ltd, Kyoto, Japan) analytical column with ambient temperature was used for the estimation. The mobile phase consisted of water and acetonitrile in 30:70 (%v/v) proportions, which was run in an isocratic mode. The flow rate was maintained at 1.2 mL/min, injection volume is 20 µL and the UV detector was set at 232 nm with run time of 8 min. The Chromeleon software was used for the analysis of results. The method was partially validated as per International Council for Harmonisation (ICH) guideline Q2 (R1).[8]

Analytical Method for Analysis of Degradation Product

The HPLC based stability indicating method for the related compounds (drug degradation) of CTX was developed. The HPLC system was comprised of Agilent 1100 Series (Agilent Technologies, California, USA) with a gradient pump, an auto sampler unit and a UV detector. YMC Pack Pro C18 RS 5µ, (250 mm × 4.6 mm) (YMC Co. Ltd, Kyoto, Japan) analytical column, operated at column temperature of 60^0C. The mobile phase consisted of two solvent systems i.e. water and mixture of acetonitrile and methanol (80:20 %v/v) run in gradient mode as shown in below Table 1. The flow rate was maintained at 1.2 mL/min, injection volume is 20 µL and the UV detector was set at 232 nm with run time of 65 min. The Chromeleon software was used for the analysis of results. The method was partially validated as per ICH guideline Q2 (R1).[8]

Analytical Method for Particle Size Analysis

Particle size is a fundamental quality attribute of any nanoformulation system and hence need to be critically monitored during formulation development and subsequent stability evaluation. Therefore, the instrument used for particle size measurement (Nicomp 380 ZLS from Particle Sizing Systems, PA, USA) was calibrated by measuring accuracy and repeatability of using known Polystyrene standard. Certified Polystyrene Standard of mean diameter

Table 1: Gradient program for analytical method used in quantification of related substance

Time (Min.)	*Mobile phase A%*	*Mobile phase B%*
0.0	60	40
25.0	40	60
30.1	40	60
42.0	10	90
45.0	10	90
50.0	00	100
60.0	00	100
61.0	60	40
65.0	60	40

92 ± 3 nm (NanosphereTM Size Standard, Catalogue No. 3090A, ThermoFischer Scientific, MA, USA) was used for calibration purpose. After satisfactory calibration, mean particle size and particle size distribution of CTX NPs was measured using at a detection angle of 90° at 25°C taking refractive index value of 1.333 and viscosity of 0.933 centipoises.

%Drug Association

Percentage of drug association with the nanoparticle was determined using size exclusion chromatography following the reported protocol.[9] Size exclusion chromatography worked on the principle for molecular size. In this, small molecules diffuse into the pores and their flow through the column is retarded according to their size (eluted later), while large molecules do not enter the pores and are eluted first in the column's void volume. Briefly, 500 µL of reconstituted CTX NPs (2 mg/mL) was loaded on Sephadex™ G-25M PD-10 column, which was previously equilibrated with saline solution. The column was then eluted with 0.9% sodium chloride solution by collecting small fractions (~500 µL). First 1.5 mL fraction forms void volume and may contains unentrapped drug. Fractions containing the NPs dispersions were collected, pooled and volume was measured (Test Sample). For control sample, 500 µL of reconstituted CTX NPs (2 mg/mL) was diluted with 0.9% sodium chloride solution to create concentration similar to test sample. The eluted test and control samples were analyzed using previously validated HPLC for the drug content. Percentage Associated CTX was calculated using the formula:

$$\%\text{Associated CTX} = \frac{(\%\text{ Content of CTX in Test sample}) \text{ X } 100}{\%\text{ Content of CTX in Control sample}}$$

Evaluation of Nanoparticulate System of CTX for Different Studies

In-vitro Release Study

Method for *in vitro* release study was in-house developed based on our previously work.[9] Dialysis membrane of 110 kD cut-off (from HiMedia Laboratories Pvt Ltd, Mumbai India) was used to study vitro release of CTX from CTX NPs. USP Type- II Dissolution Test Apparatus (Electro Lab TDT-08L, Mumbai, India) maintained at 37°C forms a part of the set up. Hydroxyl propyl cellulose (HPC) solution (0.005 %w/v) was prepared and adjusted to pH of 4.5 using orthophosphoric acid. In order to achieve sink condition, the prepared HPH solution (0.005% with pH 4.5) solution was mixed with ethanol in a ratio of 9:1 and used as release media. Reconstituted CTX NPs 2 mg/mL was appropriately diluted with 5% dextrose to yield solution with CTX concentration of 0.3 mg/mL. Prepared samples (0.5 mL) was filled into dialysis membrane (measuring 7 cm length) and tightly closed with universal closure to prevent any leakage from dialysis sac. Universal closure with sac was fix dissolution paddle with help of thread and apparatus was initiated immediately. At the end of time points of 0.5, 1, 2, 4, 8, 12, 16, 20, 24, 30, 36 and 48 hours, the sample (5 mL) was withdrawn and replaced with fresh release medium pre-equilibrated at 37°C. The release profile of prepared CTX NPs formulation was compared with marketed formulation (Jevtana). CTX quantification for release samples carried out by validated HPLC described previously. Data was expressed as percentage of cumulative CTX release versus time.

Differential Scanning Calorimetry (DSC)

The DSC thermograms of CTX, SPC, monosodium citrate, sucrose and optimized lyophilized CTX NPs were recorded on DSC (Q2000, TA, New Castle, USA), with precision and thermal accuracy of ± 0.05% and ± 0.01°. Each sample (2-4 mg) was heated in an aluminum pan at a scanning rate of 1°C/min in an atmosphere of nitrogen gas (40 mL/min) in the range of -20–240°C. The DSC was calibrated for baseline with empty pans, and for temperature and enthalpy with indium.

Powder X-Ray Diffraction (PXRD)

The PXRD patterns of CTX, SPC, monosodium citrate, sucrose and optimized lyophilized CTX NPs were recorded on X-ray diffractometer (Bruker D8 Advance Diffractometer, Germany). The X-ray source was a Cu K2 α tube (wavelength 1.5406 Å) operated at 40 kV and 30 mA. The samples were scanned from 3° to 50° 2Θ at a scan rate of 0.5° 2Θ sec^{-1}

Compatibility Studies

Compatibility of bulk formulation during manufacturing stages is prerequisite to ensure that product being manufactured does not interact with equipment/facility such glass, stainless steel, Rubber Tubing, and filter. Therefore, compatibility studies of bulk CTX NPs were carried out with Type I glass, SS 316L stainless steel (which is commonly used in pharmaceutical industry), rubber tube (used for high pressure homogenization operation) and filter (used in sterilization of nanoformulation prior to filing operation). Nanoformulation samples were stored/ circulated in contact with aforementioned glassware/ tanks/rubber tubing/filter and analysed after 0, 8 and

24 hours. Any change in critical quality attributes such as appearance, pH, particle size, assay and impurities were considered as unacceptable.

Photo Stability Study

The Photo stability of a product is carried out as per ICH requirement to determine the photo stability of the product.[10] As per ICH requirement, the product should be exposed to 1.2 million lux hour of fluorescent light and not less than 200 watt hours/square meter of UV light. Samples of lyophilized CTX NPs were exposed to 1.2 million lux hours of fluorescent light and 200 watt hours/square meter of UV light in Photo stability chamber. The vials were kept for exposure as per the following Plan:

a) Naked (Glass vials –type 1 clear glass) – primary pack
b) Proposed market pack - secondary packing
c) Double aluminum wrapped control samples
d) Control samples at 25°C/60% RH kept as control to check the impurity due to the temperature effect.

Thermal Cycling Study (Freeze Thaw Study)

During transit of product, it may be exposed to some adverse condition. Hence, it should be proved that the product quality does not get affected if product is exposed to this kind of adverse temperature conditions. In freeze thaw study, the product is frozen at -20°C and suddenly it would be given temperature shock by exposing the frozen samples to accelerated temperature condition of 25°C. By doing so the robustness of product would be established. The freeze thaw study was carried as per the details given below Table 2.

RESULTS

Evaluation of Nanoparticulate System of CTX for Different Studies

Particle Size

Mean particle size of prepared CTX NPs 40.6 ± 5.5 nm with PDI of 0.311 ± 0.014 was observed triplicate measurements by taking volume weighted Gaussian distribution. Fig. 1 captures a representative example of CTX NPs particle size distribution.

Data suggest, formed delivery system are true nanoparticulate system. 100% particles of drug delivery systems are in nm range.

In-vitro Release Study

Comparison of in vitro release profiles for CTX NPs vis-à-vis Jevtana (marketed formulation) is shown in Fig. 2. *In vitro* release study indicate that sustain release pattern with more than 80% of drug is release within 24 hrs. On the other hand, JEVTANA formulation exhibited 98.2 ± 2.1% drug release within first 30 minutes, indicating rapid release profile.

Slow drug release of nanoparticulate system give advantage of passive targeting of the delivery system and accumulation in to tumour over conventional available therapy.

Solid-state Characterization of Lyophilized CTX NPs

Solid-state characterization of lyophilized CTX NPs was carried out using PXRD and DSC analysis. DSC study was performed to understand the physical state of the drug (amorphous or crystallize or semi crystalline) in lyophilized CTX NPs formulation. CTX exhibits no sharp melting peak (endotherm) signifying amorphous nature, which can be further confirmed by PXRD analysis. In DSC analysis of SPC, the sharp endotherm peaks at 75.40°C and 106.02°C

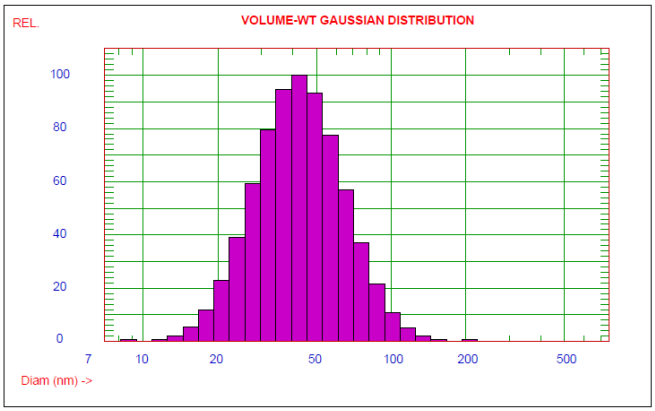

Fig. 1: Particle size of reconstituted CTX nanoparticles analysed by Nicomp 380 ZLS

Table 2: Protocol for Freeze-Thaw study

S. No.	*Temperature condition*	*Time duration*	*Physical observation*
First Cycle			
1.	-20° ± 5°C	2 days	A off-white colored lyophilized cake in a clear glass vial
2.	25° ± 2°C/60% ± 5%RH	2 days	A off-white colored lyophilized cake in a clear glass vial
Second Cycle			
3.	-20° ± 5°C	2 days	A off-white colored lyophilized cake in a clear glass vial
4.	25° ± 2°C/60% ± 5%RH	2 days	A off-white colored lyophilized cake in a clear glass vial
Third Cycle			
5.	-20° ± 5°C	2 days	A off-white colored lyophilized cake in a clear glass vial
6.	25° ± 2°C/60% ± 5%RH	2 days	A off-white colored lyophilized cake in a clear glass vial

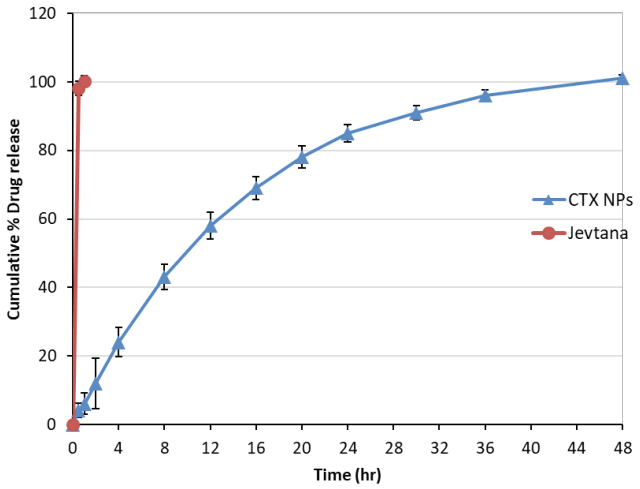

Fig. 2: Comparison of *in vitro* release profiles for CTX NPs and Jevtana

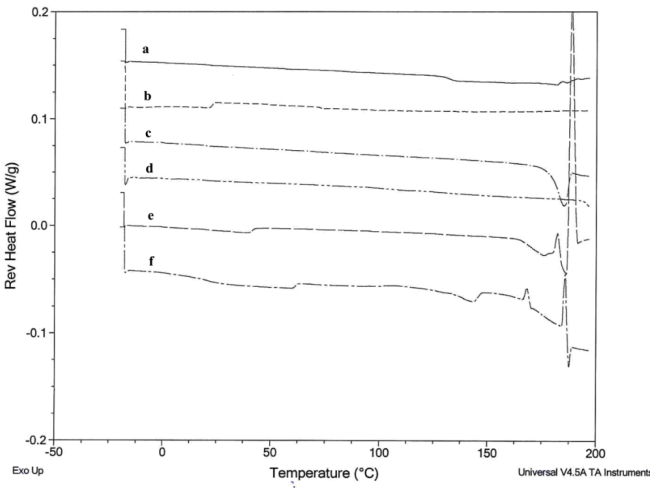

Fig. 3: DSC thermograms of (a) CTX, (b) SPC, (c) Sucrose, (d) Monosodium citrate, (e) Physical mixture and (f) Lyophilized CTX nanoparticles

were observed revealing its crystalline nature (Fig. 3). The first endotherm in SPC can be ascribed to the segmental motion associated with SPC fatty acid chains, whereas later endotherm at 106.02°C is reported to be related to the motion of polar head groups.[11]

DSC thermogram of Sucrose showed two endothermic peaks; the first small and broad peak appears at 100.18°C is related to the loss of surface adsorbed water, and another at 179.06°C attributed to melting process (Fig. 3c).[12] Melting of monosodium citrate was observed around 212^{0}C (Fig. 3d). The endothermic peak of sucrose was clearly observed in physical mixture (PM) as mentioned above but at lower temperature (Fig. 3e). This shift in melting temperature of physical mixture can be due to generation of eutectic system during the heating process.

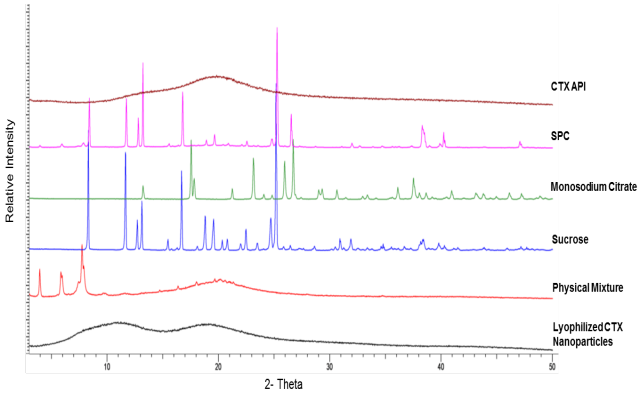

Fig. 4: PXRD spectra of (a) CTX API, (b) SPC, (c) Monosodium citrate, (d), Sucrose (e) Physical mixture and (f) Lyophilized CTX nanoparticles

The presence of exothermic peak at around 51°C followed by endothermic peak at around 149.62ºC in lyophilized CTX NPs corresponds to recrystallization of amorphous sucrose followed by melting (Fig. 3f).[12] DSC could not clearly demonstrate crystalline or amorphous nature of lyophilized CTX NPs due to ongoing heat-mediation transitions, therefore PXRD analysis was performed.

Overlay of PXRD Patterns for CTX API, (SPC, (Monosodium citrate, Sucrose, Physical mixture and Lyophilized CTX NPs are presented in Fig. 4.

CTX exhibits no sharp peaks at 2θ angle between 0° and 50° indicating its amorphous nature. The amorphous nature of CTX is reported in literature.[13] The 2θ angles of 3.957°, 5.864° and 7.755° corresponds to crystalline nature of SPC. Sucrose exhibits sharp and distinctive peaks at 2θ angles of 8.321º, 11.664º, 13.135º, 16.704º, 18.820º, and 25.184º indicating its crystalline nature (Fig. 4). The 2θ angles of 13.238°, 17.562°, 17.826°, 23.162°, 25.959°, 26.959°, 26.733° and 37.531° corresponds to crystalline nature of monosodium citrate. The characteristic peaks of SPC, sucrose and monosodium citrate were evident in physical mixture, while absent in lyophilized CTX NPs. This confirms the "amorphous" nature of lyophilized CTX NPs product.

Glass Vessel Compatibility Study

The primary objective of the Glass compatibility study is to determine the compatibility and stability of CTX bulk when kept for longer time in glass vessel during manufacturing process. Critical quality attributes of the product such as %assay, impurities levels, pH and particles size remain unchanged on 24 hrs exposures at room temperature (Table 3). This suggests the compatibility of CTX NPs with glass vessel.

Stainless Steel (SS 316L) Compatibility Study

The primary objective of stainless steel (SS 316L) vessels compatibility study is to determine the compatibility and stability of CTX NPs bulk when kept for longer time in stainless Steel vessel during manufacturing. Critical quality attributes of CTX NPs product such as %assay,

Table 3: Analysis of test parameters for glass vessel compatibility

		Storage condition		
		20–25°C (Room Temperature)		
Test	*Limits*	*Initial*	*8 Hours*	*24 Hours*
Description	Transluant liquid	Confirms	Confirms	Confirms
pH	4.0 ± 1.0	3.9	3.9	3.9
Assay, %	90 - 110	96.4	97.4	96.6
Related substances, %				
i) 7,10-Dimethoxy DAB (RRT 0.57)	NMT 0.5	0.06	0.07	0.07
ii) 7,10,13-Trimethoxy DAB (RRT 0.84)	NMT 0.5	0.03	0.04	0.04
iii) 13-Benzoyl 7,10-Dimethoxy DAB (RRT 1.03)	NMT 0.5	0.09	0.11	0.10
Single unknown impurity, %	NMT 0.5	0.04	0.06	0.05
	(RRT)			
Total impurities, %	NMT 4.0	0.37	0.45	0.43
Particle Size, µm	Mean Dia.: NMT 5 µm	28.9	29.9	26.8
	D50	25.2	26.0	23.3
Particle Size Distribution, nm	D90	50.6	53.2	48.8
	D99	89.9	95.8	90.1

Table 4: Analysis of test parameters for SS 316L vessel

		Storage condition		
		20 - 25°C (Room Temperature)		
Test	*Limits*	*Initial*	*8 Hours*	*24 Hours*
Description	Transluant liquid	Confirms	Confirms	Confirms
pH	4.0 ± 1.0	3.9	3.9	3.9
Assay, %	90 - 110	96.4	96.6	96.3
Related substances, %				
i) 7,10-Dimethoxy DAB (RRT 0.57)	NMT 0.5	0.06	0.07	0.07
ii) 7,10,13-Trimethoxy DAB (RRT 0.84)	NMT 0.5	0.03	0.03	0.04
iii) 13-Benzoyl 7,10-Dimethoxy DAB (RRT 1.03)	NMT 0.5	0.09	0.11	0.10
Single unknown impurity, %	NMT 0.5	0.04	0.06	0.06
	(RRT)			
Total impurities, %	NMT 4.0	0.37	0.46	0.45
Particle Size, µm	Mean Dia.: NMT 5 µm	28.9	28.1	27.5
	D50	25.2	24.4	23.9
Particle Size Distribution, nm	D90	50.6	50.7	49.4
	D99	89.9	92.9	90.1

impurities levels, pH and particles size remain unchanged on 24 hrs exposures at room temperature (Table 4). This demonstrate the compatibility of CTX NPs with stainless steel (SS 316L).

Rubber Tubing Compatibility Study

The primary objective of the platinum coated rubber tubing compatibility study is to determine the compatibility and stability of CTX NPs bulk when kept for longer time in Rubber Tubing during manufacturing process. Quality parameters for CTX NPs such as %assay, impurities levels, pH and particles size remain unaltered on 24 hours exposures at room temperature (20–25^{0}C) (Table 5). This shows the compatibility of CTX NPs with the platinum cured rubber tubing used in manufacturing of the CTX NPs at high pressure homogenization step.

Filter Compatibility Study

The primary objective of the surface compatibility study is to determine the compatibility and stability of CTX when in contact with various surface materials (FILTERS) used in the manufacturing process at 20–25°C temperature.

Table 5: Analysis of test parameters for Rubber tubing compatibility

		Storage condition		
		20–25°C (Room temperature)		
Test	*Limits*	*Initial*	*8 Hours*	*24 Hours*
Description	Transluant liquid	Confirms	Confirms	Confirms
pH	4.0 ± 1.0	3.9	3.9	3.9
Assay, %	90 - 110	96.4	98.1	97.6
Related substances, %				
i) 7,10-Dimethoxy DAB (RRT 0.57)	NMT 0.5	0.06	0.07	0.07
ii) 7,10,13-Trimethoxy DAB (RRT 0.84)	NMT 0.5	0.03	0.04	0.04
iii) 13-Benzoyl 7,10-Dimethoxy DAB (RRT 1.03)	NMT 0.5	0.09	0.10	0.11
Single unknown impurity, %	NMT 0.5	0.04	0.06	0.05
	(RRT)			
Total impurities, %	NMT 4.0	0.37	0.43	0.50
Particle Size, µm	Mean Dia.: NMT 5 µm	28.9	26.8	28.8
	D50	25.2	23.3	25.0
Particle Size Distribution, nm	D90	50.6	48.7	51.7
	D99	89.9	89.7	94.5

Table 6: Test parameters for filter compatibility study using PVDF 0.22 micron (Millipore)

		Storage condition		
		20–25°C (Room temperature)		
Test	*Limits*	*Initial*	*8 Hours*	*24 Hours*
Description	Transluant liquid	Confirms	Confirms	Confirms
pH	4.0 ± 1.0	3.9	3.9	3.9
Assay, %	90 - 110	96.4	97.4	96.6
Related substances, %				
i) 7,10-Dimethoxy DAB (RRT 0.57)	NMT 0.5	0.06	0.07	0.07
ii) 7,10,13-Trimethoxy DAB (RRT 0.84)	NMT 0.5	0.03	0.04	0.04
iii) 13-Benzoyl 7,10-Dimethoxy DAB (RRT 1.03)	NMT 0.5	0.09	0.11	0.10
Single unknown impurity, %	NMT 0.5	0.04	0.06	0.05
	(RRT)			
Total impurities, %	NMT 4.0	0.37	0.45	0.43
Particle Size, µm	Mean Dia.: NMT 5 µm	28.9	29.9	26.8
	D50	25.2	26.0	23.3
Particle Size Distribution, nm	D90	50.6	53.2	48.8
	D99	89.9	95.8	90.1

PVDF, PES and Nylon 66 filter membrane were assessed for the compatibility of material construction of these filter with CTX NPs. The results of study are presented in Tables 6–8. Study with PVDF, PES & Nylon 66 filters showed no significant change even at the end of 24 hrs in the assay values, the impurity profile and other critical quality attributes. This compatibility study proves suitability of PVDF, PES and Nylon 66 types of filter membrane for processing of CTX. We selected PVDF as filter for aseptic sterilization owing to its better filtration efficiency.

Overall, the compatibility studies ensure that the materials of construction of vessels (stainless steel and glass)/rubber tubing/filter being used in manufacturing of CTX NPs does not pose any risk to product's critical quality attributes.

Photo Stability Study

The photostability of exposed lyophilized CTX NPs 60 mg/vial were analyzed chemically and the results are given in following Table 9. As per the stability data, product is stable at 2 to 8°C. Impurities slightly increased at accelerated conditions (25°C/60% RH). Considering

Table 7: Test parameters for filter compatibility study using PES 0.22 micron (Millipore)

		Storage condition		
		20–25°C (Room Temperature)		
Test	*Limits*	*Initial*	*8 Hours*	*24 Hours*
Description	Transluant liquid	Confirms	Confirms	Confirms
pH	4.0 ± 1.0	3.9	3.9	3.9
Assay, %	90 - 110	96.4	97.6	96.1
Related substances, %				
i) 7,10-Dimethoxy DAB (RRT 0.57)	NMT 0.5	0.06	0.07	0.08
ii) 7,10,13-Trimethoxy DAB (RRT 0.84)	NMT 0.5	0.03	0.04	0.04
iii) 13-Benzoyl 7,10-Dimethoxy DAB (RRT 1.03)	NMT 0.5	0.09	0.10	0.12
Single unknown impurity, %	NMT 0.5	0.04	0.05	0.05
	(RRT)			
Total impurities, %	NMT 4.0	0.37	0.43	0.53
Particle Size, µm	Mean Dia.: NMT 5 µm	28.9	28.1	28.2
	D50	25.2	24.4	24.6
Particle Size Distribution, nm	D90	50.6	50.7	51.1
	D99	89.9	92.2	93.7

Table 8: Test parameters for filter compatibility study using Nylon-66 0.22 micron (Pall)

		Storage condition		
		20–25°C (Room Temperature)		
Test	*Limits*	*Initial*	*8 Hours*	*24 Hours*
Description	Transluant liquid	Confirms	Confirms	Confirms
pH	4.0 ± 1.0	3.9	3.9	3.9
Assay, %	90 - 110	96.4	97.2	95.7
Related substances, %				
i) 7,10-Dimethoxy DAB (RRT 0.57)	NMT 0.5	0.06	0.07	0.07
ii) 7,10,13-Trimethoxy DAB (RRT 0.84)	NMT 0.5	0.03	0.04	0.03
iii) 13-Benzoyl 7,10-Dimethoxy DAB (RRT 1.03)	NMT 0.5	0.09	0.11	0.11
Single unknown impurity, %	NMT 0.5	0.04	0.06	0.06
	(RRT)			
Total impurities, %	NMT 4.0	0.37	0.44	0.48
Particle Size, µm	Mean Dia.: NMT 5 µm	28.9	28.9	28.1
	D50	25.2	25.2	24.5
Particle Size Distribution, nm	D90	50.6	51.7	51.2
	D99	89.9	93.7	94.5

this impact, control samples were stored at 25°C/60% RH during the Photostability study to understand the difference of impurities levels due to light and temperature. Impurities were increased significantly in naked vials (in USP type clear glass vial) compared to double aluminium wrap vial and marketed pack vials. Naked vials data indicated that impurities increased significantly that may be due to light exposure. Total impurities were comparable between marketed pack and double aluminum pack. When marketed pack data evaluated against the control sample data, impurities levels were not increased significantly. Other analytical data were not altered in any of sample when compared to control. No considerable impact is observed on particles size distribution in all samples.

Based on analytical results, it can be concluded that lyophilized CTX nanoparticle is light sensitive in naked vial but photostable in proposed marketed pack.

Thermal Cycling Study

As shown in Table 10, the physical and chemical parameters

Table 9: Photo stability data of lyophilized CTX nanoparticles

			Postive Control	*Actual Pack*	*Negative Control*
			Primary Packing	*Secondary Packing*	*Control Sample*
Test	*Limits (Tentative)*	*Initial*	*Necked Vial*	*In Carton*	*Aluminium Wrapped*
Description	White to off-white cake	Conforms	Conforms	Conforms	Conforms
pH	4.0 ± 1.0	4.0	3.8	3.9	3.9
Water by KF, %	NMT 4.0	1.2	0.9	0.8	0.8
Reconstitution time, min	NMT 15 mins	19 Sec.	18 Sec.	18 Sec.	19 Sec.
Assay, %	90–110	99.7	78.3	98.7	98.1
Related substances, %					
i) 10-methoxy DAB (RRT 0.26)	To be recorded	ND	ND	ND	ND
ii) 7-epi-10-methoxy DAB (RRT 0.52)	To be recorded	ND	ND	ND	ND
iii) 7,10-Dimethoxy DAB (RRT 0.57)	To be recorded	0.09	0.08	0.08	0.10
iv) 7,10,13-Trimethoxy DAB (RRT 0.84)	To be recorded	0.04	0.03	0.03	0.04
v) 13-Benzoyl 7,10-Dimethoxy DAB (RRT 1.03)	To be recorded	0.09	0.14	0.08	0.08
Single unknown impurity, %	NMT 0.5	0.05	9.72	0.04	0.05
	(RRT)	(RRT)	(RRT 1.11)	(RRT 1.23)	(RRT 1.23)
Total impurities, %	NMT 4.0	0.48	11.75	0.39	0.46
Mean Dimeter - PSD, nm	Less than 200 nm	38.9	38.0	40.8	40.6
Particle Size Distribution, nm	D25	24.1	23.7	26.1	26.3
	D50	34.6	33.4	36.2	36.2
	D90	64.5	64.7	50.3	66.8
	D99	107.2	111.1	112.6	110.0

Table 10: Thermal cycling data of lyophilized CTX NPs

			3 F/T Cycle
Test	*Limits (Tentative)*	*Initial*	*2-8°C / 25°C*
Description	White to off-white cake	Conforms	Conforms
pH	4.0 ± 1.0	4.0	3.9
Water by KF, %	NMT 4.0	1.2	0.6
Reconstitution time, min	NMT 15 mins	19 Sec.	19 Sec.
Assay, %	90–110	99.7	98.1
Related substances, %			
i) 10-methoxy DAB (RRT 0.26)	To be recorded	ND	ND
ii) 7-epi-10-methoxy DAB (RRT 0.52)	To be recorded	ND	ND
iii) 7,10-Dimethoxy DAB (RRT 0.57)	To be recorded	0.09	0.07
iv) 7,10,13-Trimethoxy DAB (RRT 0.84)	To be recorded	0.04	0.03
v) 13-Benzoyl 7,10-Dimethoxy DAB (RRT 1.03)	To be recorded	0.09	0.08
Single unknown impurity, %	NMT 0.5	0.05	0.17
	(RRT)	(RRT)	(RRT)
Total impurities, %	NMT 4.0	0.48	0.62
Mean Dimeter - PSD, nm	Less than 200 nm	38.9	39.3
Particle Size Distribution, nm	D25		24.3
	D50	34.6	34.3
	D90	64.5	67.1
	D99	107.2	116.0

of product remained unaffected even after three thermal cycles hence lyophilized cake of CTX NPs are able to tolerate exersion during handling/shipment and storage.

Discussion

Previous work focused on the systemic development of CTX loaded nanoformulation using the QbD approach[14] to overcome side effects associated with conventional formulations.[15] The formulation was systematically optimized by using Central Composite Design by taking SPC concentration, PEG 400 concentration, and HPH pressure and number of HPH passes as independent variables. Particle size and PDI were identified as dependent variables.

Present study focused to challenge the formulation and its in-process stage (bulk) and to evaluate for its end patient usage. To fulfil the said purpose, quantification of drug component, particle size, surface potential, solid state characterization, *in vitro* drug release study, compatibility study, photostability study and thermal cycling study has been conducted in either in-process bulk samples or on finished dosage form. Overall results of all the studies are satisfactory. All the results are well within the acceptance criteria established before start of the study. Such systemic study and its positive results indicate the quality of the formulation which is going to be administered in cancer patient. Such high quality formulation mitigate the risk associated with administration of poor quality drugs in the patient. This work also demonstrates the approach one should follow when the drug delivery system is going to be administered in animal or human for preclinical or clinical trials. *In vitro* study results also support the previous research work which was carried out using system DoE approach. Right selection of independent and dependent factors, its level and choice of right experimental design gives you perfectly optimised drug delivery system, which is supported by such positive outcome of extensive *in-vitro* evaluation.

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

Acknowledgments

Authors would like to thank Dr. Imran Ahmad (Jina Pharmaceuticals Inc.) for his kind guidance and support.

References

1. Mohanraj VJ, Chen Y. Nanoparticles-a review. Tropical journal of pharmaceutical research. 2006;5(1):561-573.
2. Merisko-Liversidge EM, Liversidge GG. Drug nanoparticles: formulating poorly water-soluble compounds. Toxicologic pathology. 2008;36(1):43-48.
3. Cooper ER. Nanoparticles: a personal experience for formulating poorly water soluble drugs. Journal of Controlled Release. 2010;141(3):300-302.
4. US Department of Health and Human Services. Food and Drug Administration Center for Drug Evaluation and Research (CDER), Guidance for Industry Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients 2005.
5. Fusser M, Øverbye A, Pandya AD, Mørch Ý, Borgos SE, Kildal W, Snipstad S, Sulheim E, Fleten KG, Askautrud HA, Engebraaten O. Cabazitaxel-loaded Poly (2-ethylbutyl cyanoacrylate) nanoparticles improve treatment efficacy in a patient derived breast cancer xenograft. Journal of Controlled Release. 2019;293:183-192.
6. Gdowski AS, Ranjan A, Sarker MR, Vishwanatha JK. Bone-targeted cabazitaxel nanoparticles for metastatic prostate cancer skeletal lesions and pain. Nanomedicine. 2017 ;12(17):2083-2095.
7. Mathrusri Annapurna M, Venkatesh B, Naga Supriya G. A validated stability-indicating liquid chromatographic method for determination of Cabazitaxel-A novel microtubule inhibitor. J Bioequiv Availab. 2014;6:134-138.
8. Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005;1(20):5.
9. Ahmad A, Sheikh S, Ali SM, Ahmad MU, Paithankar M, Saptarishi D, Maheshwari K, Kumar K, Singh J, Patel GN, Patel J. Development of aqueous based formulation of docetaxel: safety and pharmacokinetics in patients with advanced solid tumors. Journal of Nanomedicine & Nanotechnology. 2015;6(3):1.
10. Guideline IH. Stability testing of new drug substances and products. Q1A (R2), current step. 2003;4:1-24.
11. Dhaundiyal A, Jena SK, Samal SK, Sonvane B, Chand M, Sangamwar AT. Alpha-lipoic acid–stearylamine conjugate-based solid lipid nanoparticles for tamoxifen delivery: formulation, optimization, in-vivo pharmacokinetic and hepatotoxicity study. Journal of Pharmacy and Pharmacology. 2016;68(12):1535-1550.
12. Beckett ST, Francesconi MG, Geary PM, Mackenzie G, Maulny AP. DSC study of sucrose melting. Carbohydrate research. 2006;341(15):2591-2599.
13. Lahiri S, Mishra BB, Ojha V, Panda N, Shukla SP, inventors; Fresenius Kabi Oncology Ltd, assignee. Amorphous form of cabazitaxel and process for its preparation. United States patent US 9,199,953. 2015.
14. Paithankar M, Bhalekar M. Quality by Design Enabled Development and Optimization of the Nanoparticulate System of Cabazitaxel. Int. J. Pharm. Sci. Drug Res. 2022;14(1):112-121
15. Gupta N, Yadav V, Patel R. A Brief Review of the Essential Role of Nanovehicles for Improving the Therapeutic Efficacy of Pharmacological Agents Against Tumours. Current Drug Delivery. 2021.

HOW TO CITE THIS ARTICLE: Paithankar M, Mangesh Bhalekar. *In-vitro* Evaluation and Characterization of the Nanoparticulate System of Novel Taxane Derivative. Int. J. Pharm. Sci. Drug Res. 2022;14(2):269-278. **DOI:** 10.25004/IJPSDR.2022.140218