Ind. J. Pharm. Edu. Res., 2026; 60(3s):s1024-s1036.
https://www.ijper.org

Original Article

Design of Experiments (DoE)-Based Optimization of Pomalidomide Nanosuspension for Improved Oral Absorption and Pharmacokinetics

Shaik Sabiha Thabbssum[1], Darna Bhikshapathi[2,*], Pavan Kumar Jaini[3], Medipally Viswaja[2], Mohammed Apsar Pasha[4]

[1]Department of Pharmacy, Bir Tikandrajit University, Canchipur, Imphal west, Manipur, INDIA.
[2]Department of Pharmaceutics, Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad, Telangana, INDIA.
[3]Department of Pharmacy, Raffles University, Neemrana, Rajasthan, INDIA.
[4]Department of Pharmacy, Motherhood University, Roorkee, Uttarakhand, INDIA.

ABSTRACT

Aim: The study aims to enhance the oral bioavailability and solubility of Pomalidomide (POM), which is classified as a class IV medication based on the Biopharmaceutical Classification System (BCS). **Materials and Methods:** A POM Nanosuspension (NS) was developed using a high-pressure homogenizer. The selection of stabilizers was initially done using a One-Factor-at-A-Time (OFAT) method, considering both PdI and Particle Size (PS). A Box-Behnken Design (BBD) with 3 factors and 3 levels was employed in 17 experimental trials to study how stabilizer concentration, cycle count, and pressure impact Zeta Potential (ZP) and PS. The combination of Soluplus® and Hydroxypropyl Methylcellulose (HPMC) was identified as the optimal stabilizer mix. **Results:** The resulting NS displayed a reduced PS of 132.9 nm and a ZP of -22.9 mV. SEM images showed that the particles were spherical. Further investigation with FT-IR, DSC, and X-ray Diffraction (XRD) studies substantiated the compatibility and alteration of POM into an amorphous state. The optimized POM-NS demonstrated a 21.56-fold increase in solubility, with more than 97% drug release within the first hour. The formulation also showed a 2.14-fold increase in C_{max} and a 1.56-fold increase in AUC_{0-t}. **Conclusion:** The modified POM NS markedly improved solubility, oral absorption, and bioavailability, indicating its potential for higher therapeutic efficacy.

Keywords: Pomalidomide, Nanosuspension, Box-Behnken Design, Drug Release, Pharmacokinetics.

Correspondence:
Dr. D.V.R.N. Bhikshapathi
Professor & Principal, Department of Pharmaceutics, Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad-500097, Telangana, INDIA.
Email: dbpathi71@gmail.com

Received: 09-01-2026;
Revised: 23-02-2026;
Accepted: 16-04-2026.

INTRODUCTION

The thalidomide-related compounds, the Immunomodulatory imide Drugs (IMiDs), have been well-repurposed to treat multiple myeloma, psoriatic arthritis, and Kaposi sarcoma, among other related conditions.[1] Of late, as the use of these compounds in Neurological Disorders (ND) involving inflammatory and neuroinflammatory processes has gained momentum, the scenario for potential treatments has opened up in this field.[2,3] This interest is mainly due to the diverse effects of IMiDs, especially their potent anti-inflammatory capabilities that arise from their ability to inhibit pro-inflammatory cytokine production.[4]

Chronic neuroinflammation is a common feature of many ND and an important aspect in the pathogenesis of ND, such as Parkinson's Disease (PD) and Alzheimer's Disease (AD).[5] Therefore, by targeting inflammatory pathways, IMiDs have a hopeful approach to treating the neuroinflammatory components of both neurodegenerative and psychiatric conditions.

POM is another third-generation IMiD that recently received FDA approval as a therapeutic agent for multiple myeloma and is sold under the name Pomalyst. This drug possesses highly effective anti-TNF-α action, accomplished at a much lesser dose than its antecedent, thalidomide. Moreover, POM has less risk for teratogenic effects, anti-angiogenic action, and decreased neurotoxicity than thalidomide, as shown in animal studies. Its mechanism of inhibiting TNF-α works through post-translational regulation, effectively suppressing the inflammatory response.[6,7]

In addition, POM has been found to decrease inflammation-related neuronal damage in cellular and animal models of neurodegenerative diseases and stress.[8] Very recently, it has been shown that POM is neuroprotective in a Drosophila model of PD correlated with the LRRK2 gene mutation. Furthermore,

DOI: 10.5530/ijper.20262826

POM's ability to alter disease progression has been observed in pathological models of PD based on α-synuclein in rats and rodents suffering traumatic brain injury; this shows the wide therapeutic scope of POM in neuroprotection and neuroinflammation management.[8,9] POM has an excellent CNS MPO score and good brain partitioning but poor gastrointestinal permeability due to low water solubility with negligible or minimal lipophilicity that is independent of pH level.[10] Therefore, POM falls into Class IV of BCS with poor solubility and limited permeability, hence, its oral bioavailability is significantly restricted; this could have irregular and suboptimal absorption.[11] Hence, developing it as a Nanosuspension (NS) offers a promising formulation approach to improve its dissolution rate and membrane transport. This formulation strategy aims to improve oral absorption and pharmacokinetic performance, thereby maximizing therapeutic efficacy.

The studies based on evaluations of POM's oral bioavailability showed that the value at a dose of 100 mg/kg in rats and monkeys is notably low, between 13% and 15%. However, a much higher bioavailability of nearly 100% was observed at a much lower dose of 2 mg/kg in monkeys (European Medicines Agency, 2019), which further indicates that the absorption of the drug is seriously limited by its solubility. Consequently, enhancing the dissolution rate and solubility of POM is crucial in surmounting the problems the class faces concerning absorption within the gastrointestinal tract.

Among the strategies developed to enhance BCS Class IV drug bioavailability, one approach to increase the solubility and permeability is using NS as a nanosizing has emerged as one of the best methods.[12] A NS is a colloidal system containing particles of the drug less than one micron in size, stabilized by surfactants or polymers in either an aqueous or non-aqueous medium.[13] In this formulation, drugs with poor water solubility have their solubility and bioavailability increased, thereby making them easy to deliver via oral, parenteral, and topical routes.

The manufacture of NS is fairly simple, scalable, and inexpensive. Therefore, drug formulation into nanocrystals is of high promise since it provides a set of unmatched benefits: enhanced solubility, better dissolution profile, improved bioavailability, and reduced variability in absorption under different conditions: fasted or fed.[14] There are two most commonly used approaches for NS preparation. The first is the bottom-up approach, and the second is the top-down approach.[15] Bottom-up can be feasible from the laboratory point of view of simplicity, cost-effectiveness, and lack of the need for specialized equipment; however, it always offers industrial applicability. The top-down principle incorporates the reduction of larger particles into smaller pieces through high-energy techniques such as High-Pressure Homogenization (HPH) or milling.[16] Contrarily, the bottom-up method generates nano-sized particles by precipitation of dissolved drugs. However, the main drawback of the bottom-up approach is that it is challenging to control its size, and non-uniformity and instability persist in the bottom-up approach.

In 2022, nanocrystals of POM were reported by Maria Cristina Cardia using wet media milling using Tween 80 as a stabilizer. The authors reported that the solubility increased, and there were increased levels of the drug in plasma and brain. One limitation of wet media milling is the possible contamination of the product by balls, and not suitable for industrial scale.[17] This research focused on formulating and assessing a POM NS using HPH to achieve nanosized particles with enhanced solubility, improved dissolution rates, and favorable pharmacokinetic characteristics. The formulation was developed using a 3-level, 3-factor Box-Behnken Design (BBD), with Soluplus® and HPMC as stabilizing agents. The Particle Size (PS) and Zeta Potential (ZP) of the NS were assessed using Dynamic Light Scattering (DLS) within one hour of being produced, with the stability of the NS monitored for three months. The solubility and dissolution properties of the formulation were analyzed in a PB of pH 7.4 (phosphate buffer) at 37°C. Scanning Electron Microscopy (SEM) was utilized to examine the morphology of the NS. Rats were utilized in experiments conducted in living organisms to assess the drug's plasma concentrations in both the formulated and unformulated versions.

MATERIALS AND METHODS

Materials

POM was a gift sample provided by Hetero Laboratories, a private limited company in Hyderabad, India. Soluplus® was supplied by TCI Chemicals, India. Polyvinyl pyrrolidone K30 (PVP K30), Sodium Dodecyl Sulphate (SDS), and Tween 80 were supplied by SD Fine Chemicals, Hyderabad. Pluronics F 127 was a gift sample from BASF. Mannitol and HPMC E-15 have been obtained from Sigma Aldrich, India. The solvents utilized were sourced from Merck in India.

Methods

Preparation of NS using HPH

The NS preparation involved suspending 100 mg of the drug in water-containing stabilizers: Soluplus®90 and HPMC. After dissolving the chosen stabilizers in 100 mL of water, the medication was evenly distributed on an IKA magnetic stirrer set at an rpm of 800 rpm (15 min). Later, the microsuspension was subjected to high-shear homogenization at a speed of 20,000 rpm for 15 min to decrease P.S. under 5 microns. The NS was finally processed with five cycles of Microfluidics (HPH) at 1500 bar. Then, mannitol cryoprotectant (1% w/v) was added, and freeze-drying was done for long-term storage using lyophilization by the Skadi-Europe, Model no: FD5508.[18]

In the formulation development of POM-NS, a thorough and systematic approach of prescreening followed by application

of Quality by Design was taken up as the overall assessment of the formulation and process factors involved in the final product of NS is highly desirable. QbD (Quality by Design) was thus applied in developing a POM-loaded NS.

This formal approach with well-defined objectives has been immensely supported and recommended by regulatory bodies during drug product development. QbD has found immense acceptance and appreciation among the industries and scientific communities over the last decades.

The ICH Q8 defined the goals in having a proper understanding of risk by using prior understanding, application of Design of Experiments (DoE), and robust statistical techniques throughout the life cycle of a product (R2). That is in contrast with the regular way of doing things, as the QbD framework not only ensures the cost-effective quality of a product but also offers additional insight into its manufacturing process.[19]

The (FbD) formulation-by-design approach initiates with creating a Quality Target Product Profile, a core part of the process; it is often termed as the "goal or objective setting" under the concept of FbD. QTPP refers to establishing necessary objectives and characteristics that are important for the product's quality, safety, and effectiveness.

This step is followed by fine-tuning to recognize Critical Formulation Attributes (CFA) and Critical Process Parameters (CPP). The process involves assessing the different variables involved in the formulation and the process that can impact the Critical Quality Attributes (CQAs) identified. CFA describes formulation-associated factors affecting CQAs, whereas CPPs are associated with process-related factors. The design space is part and parcel of the step to be followed, further depicting the feasible and infeasible operating regions along with their constituent variable combinations. More importantly, regulatory authorities treat activities carried out in such a design space as compliant rather than considering them as deviations.[20]

The experiment design is regarded as a structured process to manage the effects of input features on CQAs.[21] Before the Box-Behnken Design (BBD) was carried out, risk assessment tools like the Ishikawa (fishbone) diagram were used to systematically find and group possible sources of variability in the formulation and process parameters of the POM nanosuspension. The analysis recommended identifying the critical elements that were most likely to impact important quality attributes, such as processing cycles, stabilizer concentration, and homogenization pressure. This guaranteed the efficiency and focus of the formulation optimization study.

A BBD reduces the sample size required for estimation using an incomplete block and exponential method. Table 1 outlines the three factors used in the BBD to estimate the effects of independent variables, specifically (A) stabilizer-to-drug ratio ranging from 10% to 40%, (B) number of cycles varying between 1 and 5, and (C) pressure levels from 500 bar to 1500 bar, on PS and ZP at three different levels. The analysis was executed using Response Surface (SR) Charts and Contour Plots (CP) created with Design Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN).

Using the desirability function helps increase the probability of getting an optimal formulation. Based on target values, the desirability score ranges from zero to one, with scores nearer 1 indicating a significantly higher likelihood of getting the intended outcomes. Furthermore, inside the design area, pictorial optimization was done. To validate the design, a checkpoint investigation was used. The data received and the projected results were compared following three confirmation runs.

Checkpoint analysis was applied for the validation of the design. After three confirmatory runs, the obtained outputs were compared with the predicted outputs.

Cryoprotectant Screening

The NS was prepared via HPH in water. The aqueous system's thermodynamic drive increases surface area, creates free energy, and decreases interfacial energy, promoting aggregation. The stability, as well as the final product's shelf life, increases with lyophilization since the product will be kept in a more stable dry form; it must first be reconstituted with water before application. In the case of an NS freeze-dried in the presence of correctly selected cryoprotectants, its integrity proves to be a crucial factor affecting the efficiency of freeze-drying. Mannitol, trehalose, and sucrose were investigated in this study as cryoprotectants at a series of concentrations from 1% to 4% (w/v).[22]

HPLC method establishment

Quantitative analysis was performed using an HPLC instrument containing a Waters 2996 PDA detector and a 2693 pump associated with an automatic injector with a 20-µL injection volume. Separation was achieved using a 5 µm XTerra RP-C18 column with 250 × 4.6 mm as column dimensions and was controlled using Empower 2. The system operated continuously for half an hour at a constant flow rate of 0.7 mL/min, with the column temperature held steady at 40°C. The mobile phase consisted of acetonitrile and 0.03 M KH_2PO_4 (in a 20:80 v/v ratio, pH 3.2 regulated with o-phosphoric acid). The diluent used in gradient mode was a mix of water and acetonitrile in a 50:50 volume ratio. Before injecting the sample, the solvent system underwent filtration using a 0.45 µm membrane. The PDA detector was employed for monitoring the eluents at 220 nm. A 1 mg/mL drug stock solution was created by measuring the drug and afatinib, which served as the internal standard. Subsequently, a secondary stock solution at a 100 µg/mL concentration was used to generate a calibration curve ranging from 50 to 100 ng/mL.

Sample extraction for bioanalysis

The drug was isolated using the technique of protein precipitation from plasma samples. To rat plasma (50 µL), 250 µL of acetonitrile was added and mixed by vortexing. After that, the mixture was centrifuged at 8500 rpm for 10 min to acquire the supernatant that was later chromatographed at 220 nm.

Characterization and Evaluation of NS

Measurements of PS, Polydispersity Index (PdI), and ZP

A Zeta-sizer from Malvern Instruments (UK) was used to generate data on PS, PdI, and ZP of NS by employing DLS theory.[23]

Morphology using SEM

The structure of the raw drug as well NS was analyzed by a FESEM 250. After the ion sputter coating, the sample was fixed to the aluminum pin stubs and secured by double-sided carbon tape to carry out the analysis. The sample was examined with a magnification range of 500-10,000 times and an acceleration voltage of 30 kV at an operating distance of 10 mm.[24]

FT-IR (Fourier-Transform Infrared) Spectroscopy

Spectrum scanning of FT-IR for pure drug, PM, and optimized POM-NS has been done by a Perkin Elmer spectrometer Model 1600, USA, within the range of wave number 4000-450 cm^{-1} at a resolution of 1.0 cm^{-1}.[25]

DSC (Differential Scanning Calorimetry) and XRD study

The physicochemical properties of POM, along with potential interactions with excipients, were investigated using a Differential Scanning Calorimeter (DSC-60, Japan; make: Shimadzu Corp.). To accomplish this, 3-5 mg samples of pure POM, PM, NS, and NS stored for three months were hermetically sealed in aluminum pans. The samples were then analyzed thermally within the temperature range of 50 to 400°C, under an inert atmosphere. The automatic calculation determined the Melting Point (MP) and enthalpy of fusion. XRD for the raw drug, physical mix, and NS were obtained using a graphite monochromator and Ni-filtered Cu-K radiation at an operating voltage of 100 kV and a current of 40 kV. The specimens were scanned with a mean step size of 0.045° and a scanning time of 0.5 sec per step, covering a 2 to 80 degrees 2 theta (θ) range, especially from 2° to 60°.[26]

Stability studies

The ICH Q1A (R2) guideline standards were used to assess the improved drug-loaded NSPs for accelerated stability. Lyophilized materials were placed in glass vials and topped with an aluminum lid that was crimp-sealed at the same time. The vials were sealed with bromobutyl rubber plugs. These sealed samples were placed in a humidity room (Thermolab Humidity cum Photostability chamber) and maintained for 90 days (40 ± 2°C and 75 ± 5% RH). At specified intervals of 0 and 3 months, physical appearance, PS, and ZP were measured.[27]

Saturation Solubility Studies

POM, PM, and NS (5 mg of POM) were each placed in different vials containing 3 mL of water. The vials were then positioned on a rotary shaker at 25°C for 72 hr. Following this, the specimens were centrifuged using an ultracentrifuge at 15,000 rpm for 15,000 15 min at 25°C. The supernatant was clarified by using a 0.22 µm syringe filter and then analyzed for the content of the drug at 323 nm by a UV-vis spectrophotometer.[28]

In vitro dissolution testing

Dissolution testing was conducted in phosphate buffer with a pH of 7.4. In a USP type II dissolution apparatus containing 250 mL of the medium, 10 mg of the drug and the improved NS were both included. The apparatus was agitated at a speed of 50 rotations per minute and maintained at a temperature of 37°C with a tolerance of ± 0.5°C. 3 mL portions were extracted at scheduled time points. The condition of the sink was maintained by adding fresh media. A UV-visible spectrophotometer set to 323 nm was used to evaluate the samples to ascertain the drug release.[25]

Pharmacokinetic studies

The male Wistar rats utilized in the research were provided by the Nutrition National Institute. They weighed approximately between 180 to 220 g and were four to five weeks of age. The guidelines for caring for and using laboratory animals were followed in this study involving animals. The procedures received formal approval from the Institutional Animal Ethics Committee. The animals were accustomed to an animal house at an air-conditioned temperature with an RH of 40-60% for a week before the start of the study. They were then split into two sets of randomly consisting of 6 animals. The drug dispersion (0.25% w/v carboxymethyl cellulose) and the POM-NS (20 mg/kg B.W) were administered p.o. The animal blood was obtained from the retroorbital plexus (300 µL) and then transferred to sterilized test tubes with EDTA at definite intervals (1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 36, and 48 hr). A centrifuge was utilized to spin blood trials at 8500 rpm for 10 min. HPLC was utilized to process and examine the plasma samples extracted, following the technique previously mentioned. The analysis was conducted using WinNonlin to calculate Cmax, AUC_{0-48}, T_{max}, Kel, and $t_{1/2}$ without compartmentalizing the results.[23]

RESULTS

Formulation of POM-NS and stabilizer screening

NS of POM was prepared by HPH. The pressure and number of cycles used in the homogenization process were considered the critical factors that will considerably affect the size of the particles in nanosizing. In the present research, two series of

pressures were implemented: 500 bar and 1500 bar. Water-soluble polymers that suppress nucleation and crystal growth are critical to facilitating the supersaturation of the drug. They do this by attaching to newly growing crystal surfaces, preventing further growth by electrostatic or steric stabilization mechanisms. Selecting the appropriate polymer and determining the optimum stabilizing concentration to effect adequate surface coverage of the drug is critical in forming stable NSs.[29] Trials were conducted with varying concentrations of stabilizers, as it is crucial to determine the optimal stabilizer. During the stabilizer screening, the homogenization pressure was maintained at a constant 1000 bar, and the number of homogenization cycles was set to 2.

The PS of the NS prepared with various surfactants ranged from 162 ± 36.1nm to 2627.3 ± 480.3 nm and possessed a PdI ranging from 0.32 ± 0.084 to 0.62 ± 0.014. The PS in descending order is as follows: SDS > PVP K30 > F-127 > T80 > TPGS > HPMC E15> Soluplus®, as depicted in Figure 1. This clearly shows that the size of POM-NS was bigger when it was prepared with surfactants having the HLB values at the lower and higher ends. Particulate size and ZP are impacted by the nature of the stabilizer used.

HPMC E5 and Soluplus®, as polymeric stabilizers and water-soluble polymers, are essential for stabilizing the NS. Soluplus®, a copolymer made of polyvinyl caprolactam, polyvinyl acetate, and polyethylene glycol (PCL-PVAc-PEG), significantly improved the drug's solubility below its Critical Micelle Concentration (CMC).[29-31]

Using the DbF

The creation of POM-NS aimed to enhance solubility and bioavailability. As shown in the Supplementary Table 1, the QTPP was clearly defined and compared to the existing product. Quality by Design focuses on identifying and closely monitoring CQAs. The CQAs need a clear definition and must be maintained within set limits to reach the Quality Target Product Profile. Minimizing the drug's PS to the nanoscale can improve its poor water solubility. The investigation selected ZP and PS as CQAs. PdI, a dimensionless number, indicates the uniformity of the distribution of PS within a system. BBD was used with 17 runs. The data for both the dependent and independent variables are given in Table 2.

PS

PS is a crucial factor in improving the solubility of drugs whose solubility is very less. After conducting 17 experiments, the PS, PdI, and ZP were found to vary from 102.8 to 556.0 nm, with the ZP varying between -42.2 and -24.8 mV. These parameters were identified as essential quality attributes for the formulation of a POM-loaded NS. The Surface Response (SR), Contour Plots (CP), perturbation, and cube plots illustrate the influence of different factors on size, as shown in Figure 1.

The F-value of the model stands at 34.80, suggesting a mere 0.01% probability that the observed results are attributable to accidental variation. This confirms the model's **"quadratic"** nature, as demonstrated by its negligible lack of fit (4.67) and 8.52 % possibility that lack-of-fit is insignificant. Variables having a *p*-value of <0.0500 were shown to have a significant impact on the response, according to Analysis of Variance (ANOVA) analysis. Instead of the required value of 4, the model showed a high signal-to-noise ratio. Data of variables w.r.t factors F value and R^2 of PS, and ZP is provided in the Supplementary Table 2.

The interactions (A, B, C, AC, BC, and C^2) have substantial outcomes and are hence considered. Subsequently, these variables are supposed to be significant, and the regression comparison is as follows:

$$\text{Particle Size(P.S.)} = +132.92 - 23.94A - 30.45B - 24.75C - 24.75\,\text{AB} + 123.27\,\text{AC} + 42.35\,\text{BC} + 23.60A^2 + 3.13B^2 + 159.60C^2$$

The equation presented here depicts the interaction of stabilizer concentration (A), number of homogenization cycles (B), and pressure (C) with PS, considering interactions and quadratic influences. The negative coefficients of A (-23.94), B (-30.45), and C (-24.75) indicate the opposite effect, whereby increases in any of the variables would result in decreases in PS. Among the interactions, AB (-24.75) is desirous for the PS, AC (+123.27), BC (+42.35) favor positively, and hence those cases of size reduction may be mitigated. Quadratic terms indicate that high A (+23.60) level and C (+159.60) lead to a growth in the size of the particles,

Table 1: Experiment design factors (Drug:100 mg).

		Levels		
		LOW (-1)	Medium (0)	High (+1)
A	Stabilizer Concentration (w/w)	10		40
B	Cycles (numbers)	1		5
C	Pressure (bar)	500		1500
Responses		Constraints		
X_1	PS	Minimize		
X_2	ZP	Maximum		

but more strongly by C, whereas B has a very weak interaction at +3.13. This complex relationship shows that achieving the target PS is a result of careful optimization of these parameters.[32]

PdI

The PdI is a dimensionless estimate that reflects the width of the PS distribution, typically ranging from 0 to 1. In the developed formulations, PdI values ranged from point one zero two to point four eight. The proposed linear model has a statistically notable slight lack of fit, as seen by the model F-value of 6.46 (Figure 2).

With a minor lack of fit, the model suggested was "Linear". The F-value (6.47) suggests a mere 0.065% probability that the observed results are attributable to accidental variation. In comparison to pure error, there is an 81.72% possibility that lack of fit is insignificant. ANOVA analysis confirmed that variables with a *p*-value of <0.0500 influence the response significantly. The model obtained a signal-to-noise ratio (7.1788) of more than the required value of 4, meaning that it effectively explored the design space.

The interactions (A, AB, A^2, and B^2) had substantial outcomes and were hence considered. Subsequently, these variables were supposed to be significant, and the regression comparison is as follows:

$$\text{Polydispersity index (PDI)} = +0.2740 - 0.0142A - 0.0189B + 0.1406C$$

The linear equation given shows how stabilizer concentration A, the number of cycles of homogenization B, and pressure C affect the PdI. The positive coefficients for A (+0.0142), B (+0.0189), and C (+0.1406) show that the more any one of these variables

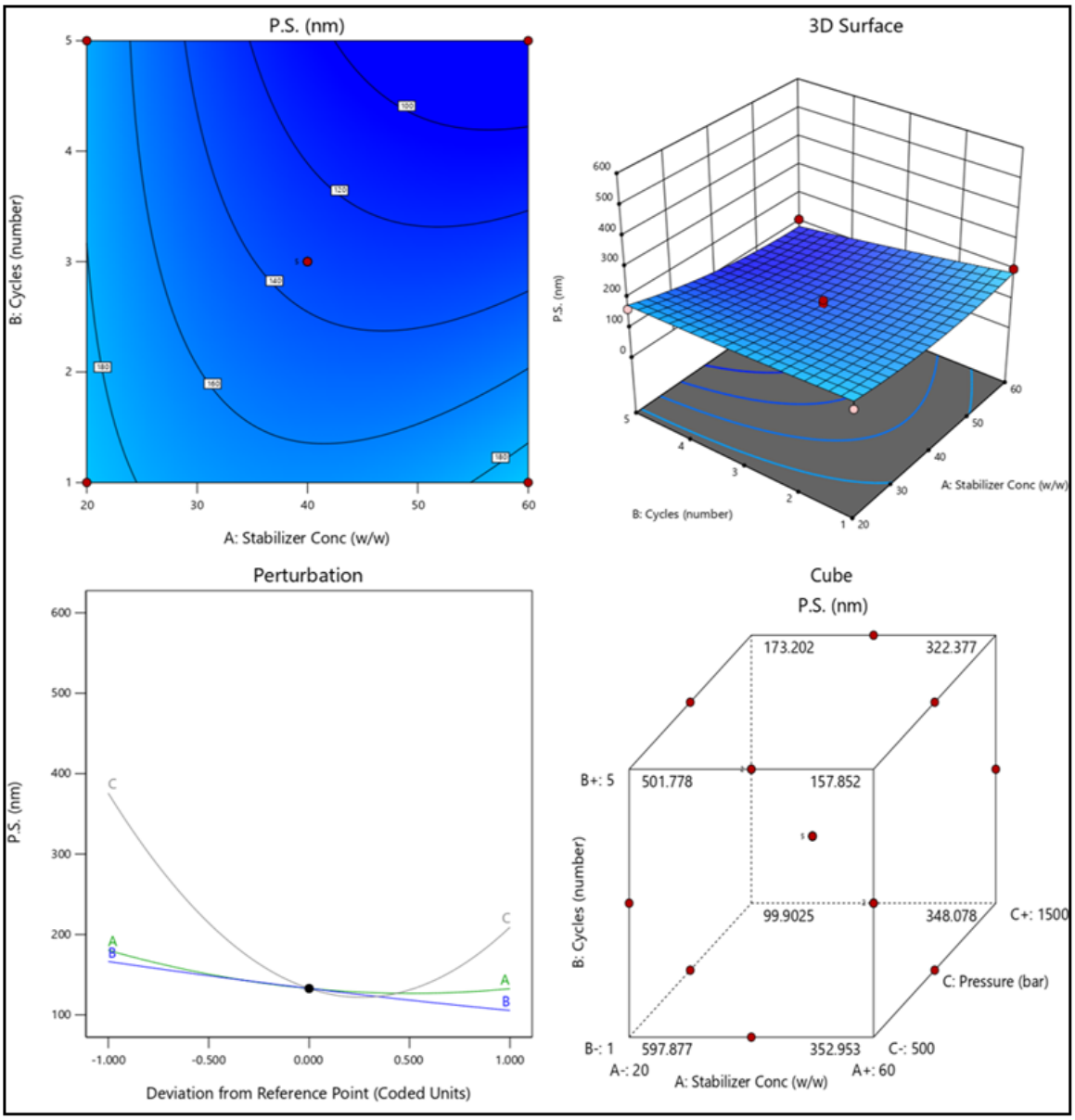

Figure 1: Graphical representation of RS, CP, PP, and cubic plots presenting the outcome of variables on PS.

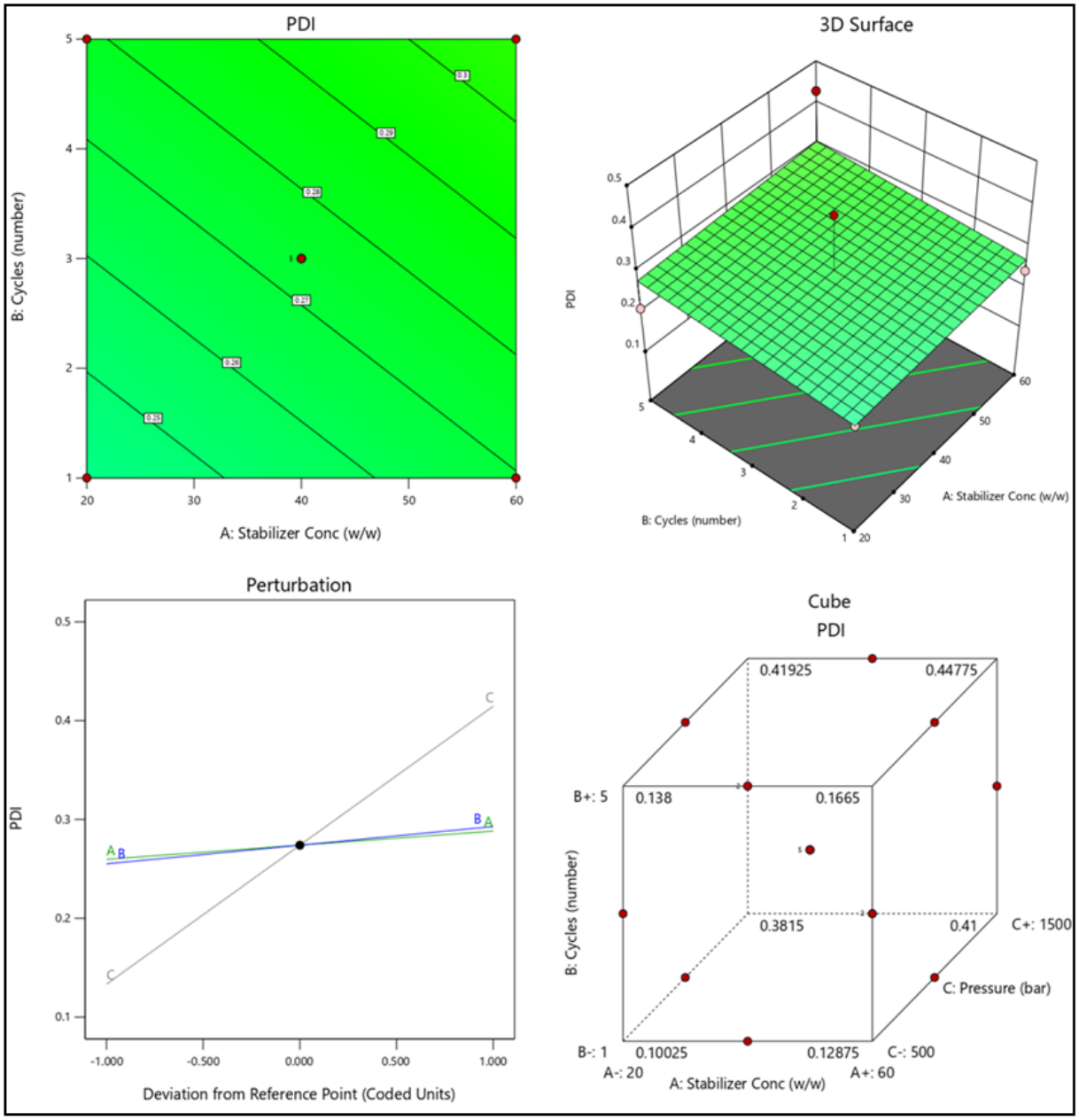

Figure 2: SR, CP, Perturbation, and cube plots depicting the outcome of variables on PdI.

increases, the higher the PdI becomes; it is under pressure C that this has the greatest effect. This would imply that high pressure is significant in contributing to increasing the variability of PS distribution, while stabilizer concentration and homogenization cycles contribute smaller effects that are still of importance. It further makes clear why careful manipulation of these factors must go into obtaining the right PS distribution.

ZP

ZP, controlled by surface charges, plays a key role in drug delivery by influencing the stability of nanoparticle suspensions and the initial interaction of nanoparticles with cell membranes. We have observed that the ZP varied from -42.2 to -24.8 mV.

The F-value of the model stands at 61.49, suggesting a mere 0.01% probability that the observed results are attributable to accidental variation. This confirms the model's "Linear" nature, as demonstrated by its negligible lack of fit (0.72) and a 68.50% possibility that lack-of-fit is insignificant. Variables having a *p*-value of <0.0500 were shown to have a significant impact on the response, according to ANOVA analysis. Instead of the required value of 4, the model showed a high signal-to-noise ratio (24.098) (Figure 3).

The interactions (A and C) had substantial outcomes and were hence considered. Subsequently, these variables are supposed to be significant, and the regression comparison is as follows:

$$\text{Zeta Potential(ZP)} = -31.41 - 5.90A - 0.9237B - 1.64C$$

The equation above shows how stabilizer concentration A, homogenization cycles B, and pressure C impact ZP. The negative intercept, -31.41, represents the baseline ZP. The coefficients for A (-5.90), B (-0.9237), and C (-1.64) are all negative, indicating that increases in stabilizer concentration, homogenization cycles, or pressure continue to decrease the ZP. One factor acts positively at a greater level than the others do; these are stabilizer concentration (A) and pressure (C), whereas homogenization cycles (B) have a minor effect. Lower ZP values further point towards good stability of the colloids, emphasizing that these parameters play a critical role in stabilizing the NSs.

Investigation for improved formulation

The design used the desirability function in numerical optimization. The formulation achieved the optimal F_{opt} solution with the highest desirability value of 0.905. The ideal parameters should be: a ratio of stabilizer-to-drug of 20; that is, 100 mg of Soluplus and 100 mg of HPMC for each 100 mg of the drug; 3.51 homogenization cycles; and a pressure of 1452 bars (Figure 4). Three checkpoints were taken into consideration for the validation to offer robustness of the model and accuracy of the formulation. In Supplementary Table 2, the data of predicted and actual mean measurements were given, and the differences in the very least.[33]

Characterization and evaluation of NS

HPLC analysis

The sample was checked by HPLC using a reverse-phase column, with a PDA detector set to 220 nm. The mobile phase consisted of 0.03 M KH_2PO_4 (adjusted to pH 3.2 with o-phosphoric acid)

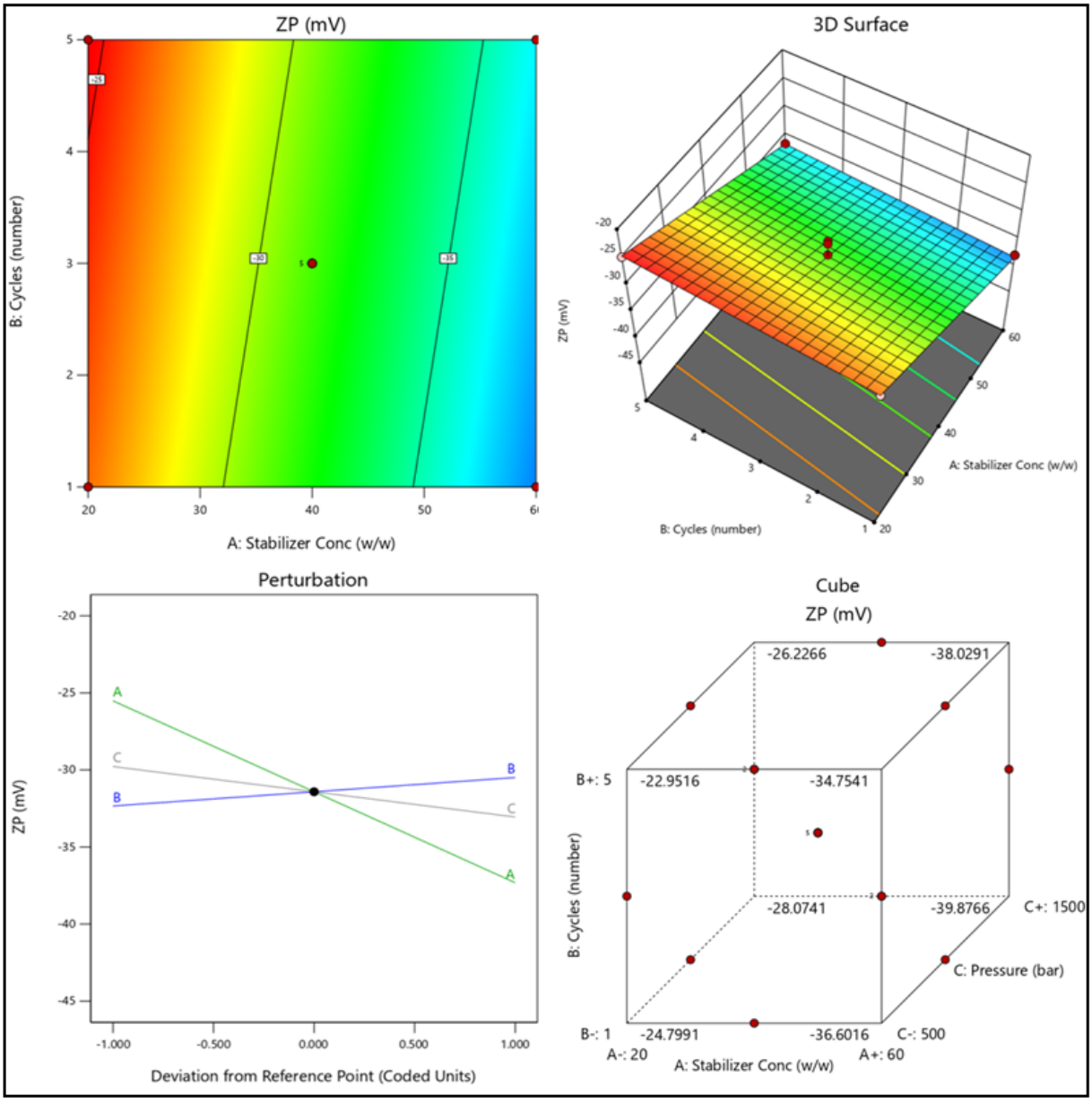

Figure 3: Graphical representation of SR, CP, perturbation, and cube plots demonstrating the consequence of variables on ZP.

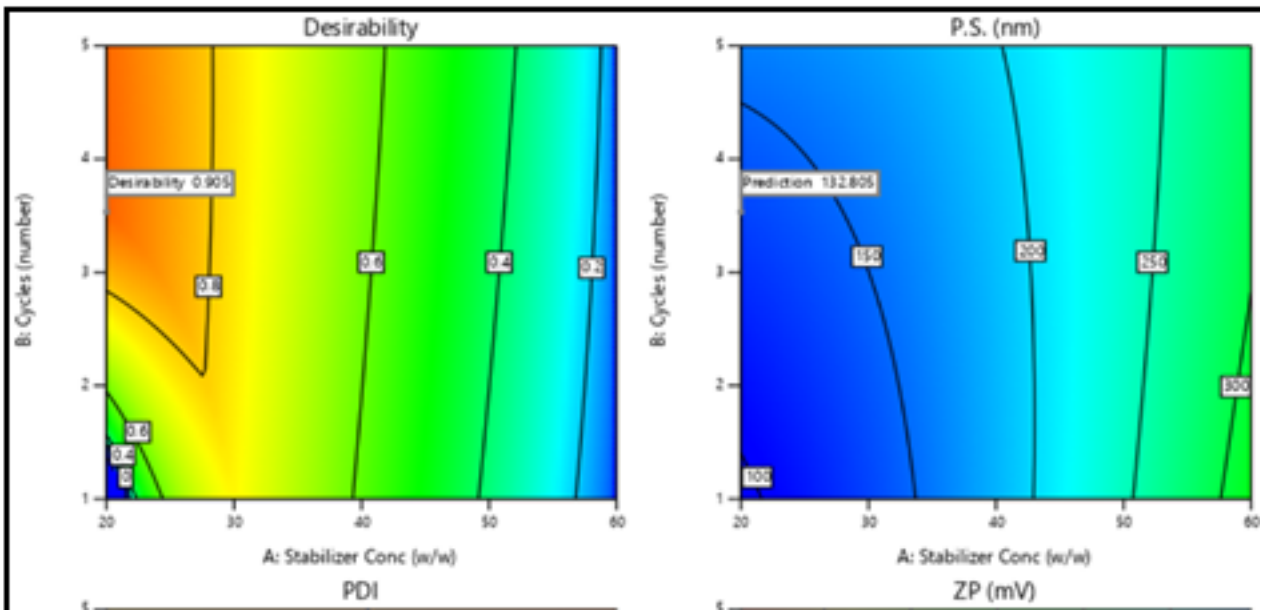

Figure 4: A graphical depiction of desirability and the overlay plot.

and acetonitrile in a 20:80 v/v ratio. A regression coefficient of 0.997 was achieved within the drug concentration range of 50 to 1000 ng/mL. Percent recovery was calculated by evaluating the analyte's response in the biological matrix relative to the pure standard.

PS, PdI, and ZP

The size of NS remained consistent, exhibiting a PS of 132.9 ± 1.4 nm and a PdI of 0.393. A PdI below 0.4 indicates a homogeneous system. The stability of NS is reflected by measuring the ZP, a property correlated with the Double Electric Layer (DEL) on the particle surface.[34] The Z.P. of the POM-NS was -22.9 ± 0.016 mV.

Morphology using SEM

The surface morphology of the formulation is assessed using SEM. Initially, the unadulterated medication displays a wide variety of PSs, including unique, random, cubic-shaped particles in the micrometer range. However, through HPH, the drug was transformed into spherical nanoparticles of uniform size distribution in the nanometer range within the NS.

FT-IR Spectroscopic analysis

The POM exhibited distinct peaks in its spectrum, which signify the existence of specific functional groups. The key groups include NH_2 stretching at 3481 and 3377 cm^{-1}, H-C=C and H-C-H stretching at 3113, 2984, and 2898 cm^{-1}, and C=O stretching at 1751, 1726, 1691, and 1632 cm^{-1}. Additionally, bands appeared at 1594, 1408, and 1320 cm^{1}, signifying C=C, C-N, and C-O vibrations. Soluplus® exhibits distinct peaks at 2964 cm^{-1} and 2877, 1739, and 1644 cm^{-1}. In the FTIR spectra of POM-NS, the characteristic peaks of Soluplus® are observed at 1739 cm^{-1} (ester) and 1644 cm^{-1} (amide).[17]

DSC study

A DSC was conducted to estimate the thermal properties of the drug, polymers, and NS. The thermogram of the pure drug displayed endothermal peaks at 198.94°C and 149.12°C, confirming its crystalline structure. HPMC showed a thermal event at around 101.4°C, and also Soluplus® had a peak around 103°C. The physical mixture evidenced peaks at 88.19°C, 165.09°C, and 349.18°C. No notable dissimilarities were noted among the thermograms of the physical mixtures, the NSs, and the pure drug, except for a decrease in peak intensity for the former.[17]

XRD

The XRD patterns indicate the diffraction peaks of the drug that have appeared clearly at 2 θ scattered angles: 12.3, 14.1, 16.92, 17.3, 18.3, 24.3, 24.7, 25.6°, and 28.0°, indicating the drug is crystalline. Such peaks in the diffraction curve of the drug had also appeared earlier.[17]

Saturation Solubility Studies

Apparent solubility was measured for the optimal freeze-dried POM-NS, containing 10 mg of POM and 2 mg of a stabilizer combination of Soluplus and HPMC. The solubility values obtained were 69.88 ± 2.12 µg/mL for the NS, 5.1 ± 0.78 µg/mL in the physical mix of the drug and stabilizer, and 3.24 ± 0.072 µg/mL for the plain drug.[35]

In vitro dissolution testing

The dissolution profile of the NS and Plain Drug (PD) was evaluated in a phosphate buffer at pH 7.4, with the release pattern. The Plain Drug (PD) exhibited a drug release of only 8.84 ± 3.78% within the first hour, whereas the NS achieved a release of 97.45

Table 2: Runs designed for the trails.

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Run	Stabilizer concentration (w/w)	Cycles (number)	Pressure (bars)	PS (nm)	PdI	ZP (mV)
1	40	1	1500	196.2	0.43	-34.8
2	40	3	1000	144	0.18	-31.1
3	20	3	1500	163.7	0.48	-27
4	20	1	1000	163.2	0.24	-26.5
5	20	3	500	556	0.18	-25.8
6	60	3	500	222	0.11	-36.9
7	40	3	1000	133.2	0.2	-31.6
8	40	1	500	468.6	0.197	-30.6
9	20	5	1000	164.4	0.21	-24.8
10	60	5	1000	106.6	0.43	-36.36
11	40	3	1000	102.8	0.405	-29.13
12	60	1	1000	204.4	0.242	-37.85
13	40	3	1000	146.1	0.19	-28.6
14	40	5	500	310.4	0.18	-28.4
15	40	5	1500	207.4	0.44	-32.8
16	60	3	1500	322.8	0.442	-40.2
17	40	3	1000	138.5	0.102	-31.6

± 3.73%. This led to a decrease in drug release from the plain drug but an increase in release from the NS. Due to polymers enhancing wettability and reducing PS, NSs significantly boosted the dissolution rate.[36,37]

Stability studies

The stability of the formulation was assessed by noting the PS and ZP throughout three months. Until the 60th day, there was minimal difference in PS among all stability settings. When stored under refrigeration (2-8°C), the size increased from 132.9 ± 1.4 nm on day 0 to 144.9 ± 3.4 nm by day 90. At the highest temperature of 40°C, the PS drastically rose from 132.9 ± 1.4 nm on day 0 to 156.12 ± 8.90 nm by day 90, suggesting a gradual loss of stabilizing integrity.[38]

Pharmacokinetic studies

The NS formulation showed significantly higher values for T_{max}, C_{max} (**$p < 0.001$), AUC_{0-24} (**$p < 0.001$), and $AUC_{0-\infty}$ (**$p < 0.001$) compared to the pure drug suspension at equivalent dosage, based on the pharmacokinetic data. The C_{max} increased significantly from 2028.02 ± 260.92 ng/mL (PD) to 4340.44 ± 380.25 ng/mL (NS), indicating increased peak plasma concentration and faster action due to faster solubility and absorption. NS had significantly higher AUC_{0-t} and $AUC_{0-\infty}$ values than PD (15882.5 ± 530.2 ng·h/mL and 18447.69 ± 794.41 ng·h/mL), despite T_{max} remaining consistent at 3 hours. The elimination half-life ($t_{½}$) of NS (5.20 ± 1.26 hr) was significantly lower than that of PD (9.74 ± 2.44 hr), possibly due to increased absorption and plasma concentrations, which may improve drug clearance. The elimination rate constant (Ke) for NS (0.133 h^{-1}) was nearly twice that of PD (0.071 h^{-1}), supporting this conclusion. Mean Residence Time (MRT) decreased from 12.08 ± 3.01 hr for PD to 8.33 ± 2.71 hr for NS, indicating faster systemic turnover with NS. The afatinib internal standard was identified at 6.6 min, the plasma peak at 3.5 min, and the drug retention time at 5.28 min on the bioanalytical chromatogram. The optimized formulation reached a maximum level (C_{max}) 2.14 times higher, while the Area Under the Curve (AUC_{0-t}) was 1.56 times higher than the free drug. Pharmacokinetic studies showed that NS increased POM's systemic exposure compared to pure medication.

DISCUSSION

In this work, NSs are manufactured using HPH. In the preliminary screening, all the selected stabilizers were screened, and a combination of stabilizers was finally selected. And by fixing the variables, 17 runs were formulated, and their effect on PS and ZP was studied. At high pressure, a great rise in reaction temperature was observed, and to counter this, cooling down immediately in an ice bath was done during HPH. Several stabilizers were tested first, and the subsequent PSs obtained are considered. HPMC E5 and Soluplus®, as polymeric stabilizers and water-soluble polymers, are essential for stabilizing the NS.[29] Soluplus®, a copolymer made of Polyvinyl Caprolactam, Polyvinyl Acetate, and Polyethylene Glycol (PCL-PVAc-PEG), significantly

improved the drug's solubility below its CMC.[34] HPMC E5, a non-ionic polymer stabilizer, possesses numerous methoxyl and hydroxypropyl groups, enabling it to interact strongly with hydrophobic moieties and form hydrogen bonds with drugs. While HPMC E5 initially reduced the size in the formulations, it subsequently induced accumulation and observable particle sedimentation. HPMC E5 swells and provides viscosity, thereby by the formation of large floc-like particles.[31]

However, formulations prepared using a single stabilizer showed physical instability beyond three days, characterized by growth in PS and PdI, attributed to the Ostwald ripening phenomenon. Hence, a combination of anionic surfactants like Soluplus® and HPMC was selected for further optimization. It lowers the interfacial tension at the particle surface by promoting an attractive interaction between water and the surfactant, which aids in the formation of small particles within the NS. The DbF method was employed to evaluate and improve the stabilizer mixture to create a stable formulation. For the stability of NS, it is mandatory to study and interpret the solubility of the drug in the chosen stabilizer. According to the LSW theory, the concentration of the drug inside the dispersion phase considerably accelerates Ostwald ripening.[38]

It has been observed that, over time, the PS in NSs can decrease due to the occurrence of Ostwald ripening. This occurs when drug particles dissolve from regions of higher concentration (larger particles) and recrystallize in regions of lower concentration (smaller particles). The difference in concentration creates a concentration gradient, facilitating the mass transfer and subsequent reduction in PS. Research on NSs has shown that the stabilizer employed in NSs may cause crystallization, agglomeration, and particle growth. Therefore, it is advised to choose stabilizers carefully so that the stabilizer has a minimal effect on the SNB solubility. The optimized formulation incorporating Soluplus and HPMC yielded an estimated solubility of 14.65 ± 2.58 µg/mL. This suggests that the concentration of the chosen stabilizers did not significantly enhance the drug's solubility.

Optimization with the BBD has led to the development of a quadratic and linear model intended to study the important factors of PS and ZP. These formulations validate the proposed model because of the great alignment of values obtained from the software with the results.[33] Soluplus with HPMC provided an optimum Z.P since the same were adsorbed onto the particle surface and hence imparted a slight negative charge to the nanoparticle. SEM studies demonstrated that through HPH, the drug was transformed into spherical nanoparticles of uniform size distribution in the nanometer range within the NS. The lack of change in functional group peaks in FT-IR spectra of NS represents the chemical interaction between API and stabilizer, which depicts the physical adsorption of stabilizer on drug NS.

In the DSC of optimized NS, two major peaks appeared with slight shifts to lower temperatures. This may be attributed to the decreased size of the drug in its nanocrystalline state, partial drug solubilization in the stabilizer as the DSC analysis was being conducted, or significant reciprocations between the drug and stabilizer. The measured downward deviation of enthalpy signifies a partial change of the drug from crystalline to amorphous. The XRD NS is characterized by the disappearance of drug characteristic diffraction peaks, indicating that the pure drug possibly forms a molecular-level, solid-state complex in the formulation. The solubility of the drug was augmented by 21-fold, signifying the role of stabilizer while maintaining the nanolevel range.[17] Furthermore, as per Ostwald-Freundlich calculation, the reduction in the particle radius would give a more significant ratio of surface area to volume and thus the solubility.[13]

The release of the drug from NS is attributed to enhancing the wettability of polymers and reducing PS, resulting in a boosted dissolution rate. The Noyes-Whitney equation indicates that there is a direct relationship between dissolving rate and surface area, with a substantial increase in total surface area as particles move from the micron to nanometer scale. Moreover, adding hydrophilic HPMC and amphiphilic Soluplus® polymers in NS formulations significantly increased dissolution rates through enhanced surface wetting. HPMC created a hydrophilic environment that enhanced the wetting of larger drug particles. On the other hand, the hydrophobic drug particles were drawn to the vinylcaprolactam/vinyl acetate side chains of Soluplus®, while its hydrophilic PEG backbone provided steric stabilization, preventing recrystallization and aggregation.[39]

This material's dual functionality for stabilizing and improving dissolution conditions through good wettability in conjunction with HPMC contributed significantly to it. Although Soluplus® showed better wettability, its performance was not reflected in higher dissolution rates compared to NSs and physical mixtures. However, Soluplus® is an excellent stabilizer for BCS Class IV drugs that greatly improve wettability and solubilization conditions, helping drug dissolution conditions. During stability studies larger PSs were noticed after 60 days, and this may be a result of lessened surface coverage of the particle. A two-fold increase in the bioavailability of POM nanosuspension means it works better at lower doses, benefiting patients in their treatment and helping them experience fewer or no dose-related side effects. The enhanced bioavailability can be attributed to the following mechanism: The absorption of POM was enhanced due to the stabilizers adsorbed onto the nanoparticles, which could improve intestinal membrane permeability through the surface activity of Soluplus. Additionally, the interaction between the drug, stabilizer, and intestinal fluids, which contain mixed micelles of bile salts and lecithin, may play a role in enhancing the formulation's *in vivo* performance.[36]

CONCLUSION

In the current study, POM-NS was made using a homogenization technique that involved high pressure, using the DoE was carried out. Seventeen runs were conducted for the selected combination stabilizers of Soluplus and HPMC. The optimized NS has shown a size of 132.0 nm and a ZP of 22.0 mV. Compatibility studies confirmed that the drug remained stable and was well-tolerated with the chosen stabilizer. The freeze-dried NS was stable at refrigerator and room conditions up to 90 days. The release testing demonstrated that the NS released over 95% of the drug within one hour, outperforming the plain drug. A pharmacokinetic analysis further indicated that the NS revealed meaningfully higher bioavailability than the plain drug. The formulation as NS is a viable option for drugs like POM for improving the therapeutic benefits for the patient.

ACKNOWLEDGEMENT

The authors would like to thank the Bir Tikandrajit University, Canchipur, Imphal, Manipur, India, and the Department of Pharmaceutics, Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad, Telangana, India, for providing facilities to carry out the research work.

ABBREVIATIONS

BBD: Box-Behnken Design; **FT-IR:** Fourier-transform infrared spectra; **XRD:** X-Ray Diffraction; **DSC:** Differential Scanning Calorimetry; **HPMC:** Hydroxy Propyl Methyl Cellulose; **RPM:** Rotations Per Minute; **SEM:** Scanning Electron Microscopy; **DOE:** Design of Experiments; **ANOVA:** Analysis of Variance; **DLS:** Dynamic Light Scattering; **POM:** Pomalidomide; **BCS:** Biopharmaceutical Classification System; **NS:** Nanosuspension; **IMiDs:** Immunomodulatory imide drugs; **ND:** Neurological disorders; **PD:** Parkinson's disease; **AD:** Alzheimer's disease; **HPH:** High-pressure homogenization; **PVP:** Polyvinyl pyrrolidone; **SDS:** Sodium dodecyl sulphate; **FbD:** Formulation-by-design; **CFA:** Critical Formulation Attributes; **CPP:** Critical Process Parameters; **CQA:** Critical Quality Attributes; **PS:** Particle size; **ZP:** Zeta potential; **PdI:** Polydispersity index; **CMC:** Critical micelle concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTIONS

The contributions from each author are equal.

ETHICAL STATEMENT

The IAEC (Protocol No. 1447/PO/Re/S/11/CPCSEA-102A) approved the study, which adhered to CPCSEA guidelines.

SUMMARY

This study involved the development of POM NSs using high-pressure homogenisation, optimised using a BBD, with an emphasis on PS and ZP. The preliminary stabiliser screening revealed that the combination of Soluplus® and HPMC E5 is best, owing to their synergistic effects in enhancing wettability, minimising PS, and stabilising the formulation. Although individual stabilisers resulted in instability and Ostwald ripening, their combination improved physical stability and solubility. The optimised NS displayed homogenous, spherical nanoparticles, with FT-IR, DSC, and XRD studies validating physical interactions and partial amorphization of the drug. Solubility increased twenty-one times, and dissolution was markedly improved, facilitated by nanosizing and the surface activity of stabilisers. Notwithstanding considerable particle development following prolonged storage, the formulation exhibited enhanced pharmacokinetic characteristics, including elevated Cmax and AUC, ascribed to improved dissolving, wettability, and potential permeability enhancement through Soluplus®. The study demonstrates that an effectively optimised stabiliser combination can markedly improve the solubility, stability, and bioavailability of BCS Class IV medicines such as POM.

REFERENCES

1. Bristol-Myers Squibb. U.S. Food and Drug Administration approves Bristol Myers Squibb's Breyanzi (lisocabtagene maraleucel), a new CAR T cell therapy for adults with relapsed or refractory large B-cell lymphoma. Corp News. 2021;5:11-2.
2. Beurel E, Toups M, Nemeroff CB. The bidirectional relationship of depression and inflammation: double trouble. Neuron. 2020;107:234-56.
3. Jung YJ, Tweedie D, Scerba MT, Kim DS, Palmas MF, Pisanu A, *et al.* Repurposing immunomodulatory imide drugs (IMiDs) in neuropsychiatric and neurodegenerative disorders. Front Neurosci. 2021;15:656921.
4. Siegel DS, Schiller GJ, Song KW, Agajanian R, Stockerl-Goldstein K, Kaya H, *et al.* Pomalidomide plus low-dose dexamethasone in relapsed refractory multiple myeloma after lenalidomide treatment failure. Br J Haematol. 2020;188:501-10.
5. Guzman-Martinez L, Maccioni RB, Andrade V, Navarrete LP, Pastor MG, Ramos-Escobar N. Neuroinflammation as a common feature of neurodegenerative disorders. *Front Pharmacol*. 2019;10:1008
6. Vargesson N, Mahony C, Erskine L, Niven J, Greig NH, Figg WD. Reply to D'Amato, *et al.* and Zeldis, *et al.*: screening of thalidomide derivatives in chicken and zebrafish embryos. Proc Natl Acad Sci U S A. 2013; 110.
7. Moreira AL, Sampaio EP, Zmuidzinas A, Frindt P, Smith KA, Kaplan G. Thalidomide exerts its inhibitory action on tumor necrosis factor α by enhancing mRNA degradation. J Exp Med. 1993;177:1675-80.
8. Wang JY, Huang YN, Chiu CC, Tweedie D, Luo W, Pick CG, *et al.* Pomalidomide mitigates neuronal loss, neuroinflammation, and behavioral impairments induced by traumatic brain injury in rat. J Neuroinflammation. 2016;13:1-17.
9. Wager TT, Hou X, Verhoest PR, Villalobos A. Moving beyond rules: the development of a central nervous system multiparameter optimization (CNS MPO) approach to enable alignment of druglike properties. ACS Chem Neurosci. 2010;1:435-49.
10. Huang YN, Greig NH, Huang PS, Chiang YH, Hoffer A, Yang CH, *et al.* Pomalidomide improves motor behavioral deficits and protects cerebral cortex and striatum against neurodegeneration through a reduction of oxidative/nitrosative damages and neuroinflammation after traumatic brain injury. Cell Transplant. 2024;33:09636897241237049.
11. You CH, Lee KY, Chey WY, Menguy R. Electrogastrographic study of patients with unexplained nausea, bloating and vomiting. Gastroenterology. 1980;79(2):311-4.
12. Stern RM, Koch KL, Stewart WR, Lindblad IM. Spectral analysis of tachygastria recorded during motion sickness. Gastroenterology. 1987;92(1):92-7.
13. Keohane J, Nadel S, Gordon I, Peters MJ. The effect of dopamine on renal function in children with shock: a double blind, randomized study. Crit Care Med. 2005;33(2):466-72.
14. FDA. Center for Drug Evaluation and Research. Summary Review. Application Number: 204026Orig1s000. 2013. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/204026Orig1s000SumR.pdf

15. Food and Drug Administration. FDA Drug Approval Package: Pomalyst (pomalidomide) Capsules. NDA #204026. 2013. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/204026Orig1s000TOC.cfm
16. Thalidomide. In: LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012. Available from: https://www.ncbi.nlm.nih.gov/books/NBK548162/
17. Arora S, Suresh S, Rattan V, Bhardwaj DN, Sethi N. A review of teratogenic effect of thalidomide. Indian J Dent Res. 2015;26(3):300-4.
18. Harper PS, Weatherall DJ, Crapper DR, McKusick VA, Emery AE. Genetic counseling and prenatal diagnosis. In: Oxford Textbook of Medicine. 4th ed. Oxford: Oxford University Press; 2003. p. 411-9.
19. Vargesson N. Thalidomide-induced teratogenesis: history and mechanisms. Birth Defects Res C Embryo Today 2015;105(2):140-56.
20. Tseng S, Pak G, Washenik K, Pomeranz MK, Shupack JL. Rediscovering thalidomide: a review of its mechanism of action, side effects, and potential uses. J Am Acad Dermatol. 1996;35(6):969-79.
21. FDA. Medication Guide: Pomalyst (pomalidomide) Capsules. Revised 02/2013. A vailable from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/204026s000lbl.pdf
22. Chen N, Lau H, Kong L, Kumar G, Zeldis JB, Knight R. Pharmacokinetics of pomalidomide in subjects with impaired renal function. J Clin Pharmacol. 2014;54(6):624-33.
23. Lacy MQ, Hayman SR, Gertz MA, Dispenzieri A, Buadi F, Kumar S, *et al.* Pomalidomide (CC4047) plus low-dose dexamethasone (Pom/dex) as therapy for relapsed multiple myeloma. J Clin Oncol. 2009;27(30):5008-14.
24. Cortes JE, Heidel FH, Hellmann A, Fiedler W, Smith BD, Robak T, *et al.* A phase 2 trial of pomalidomide in patients with chronic myelomonocytic leukemia Blood. 2015;125(6):789-94.
25. Daver N, Pollyea DA, Cortes JE, Smith BD, Roboz GJ, Steensma DP, *et al.* Efficacy, safety, and biomarkers of response to azacitidine and the novel agent CC-486 in myelodysplastic syndromes and acute myeloid leukemia: a phase 1/2 study. Lancet Haematol. 2020;7(10):e668-80.
26. List A, Kurtin S, Roe DJ, Buresh A, Mahadevan D, Fuchs D, *et al.* Efficacy of lenalidomide in myelodysplastic syndromes. N Engl J Med, 2005;352(6):549-57.
27. Gonzalez-González O, Ramirez IO, Ramirez BI, O'Connell P, Ballesteros MP, Torrado JJ, *et al.* Drug stability: ICH versus accelerated predictive stability studies. *Pharmaceutics*, 2022;14(11):2324.
28. Chen N, Zhou S, Palmisano M. Clinical pharmacokinetics and pharmacodynamics of lenalidomide. Clin Pharmacokinet, 2017;56(2):139-52.
29. Guembe-Michel N, Nguewa P, González-Gaitano G. Soluplus-based pharmaceutical formulations: recent advances in drug delivery and biomedical applications. *Int J Mol Sci*, 2025;26(4):1499.
30. Mali KD, Shinde DT, Patil KR. Review on Soluplus®: Pharmaceuticals revolutionizing drug delivery and formulation strategies. *Int J Pharm Sci Drug Res*, 2025;15(2).
31. Teo SK. Properties of thalidomide and its analogues: implications for anticancer therapy. AAPS J, 2005;7(1):E14-9.
32. Gandhi AK, Mendy D, Waldman M, Chen G, Rychak E, Miller K, *et al.* Measuring cereblon as a biomarker of IMiD activity and resistance. Leukemia, 2014;28(11):1988-90.
33. Ge Y, Waldman M, Grieselhuber NR, Zhou Y, Liu C, Yan H, *et al.* Quantitative assessment of cereblon protein in multiple myeloma: Correlation with IMiD response and mechanisms of cereblon regulation. Leukemia, 2022;36(1):169-77.
34. Melchert M, List A. The thalidomide saga. Int J Biochem Cell Biol, 2007;39(7-8):1489-99.
35. Lentz SR. Thalidomide and its derivatives: emerging from the wilderness. J Thromb Haemost, 2004;2(2):209-10.
36. Sumathi R, Tamizharasi S, Sivakumar T. Formulation and evaluation of polymeric nanosuspension of naringenin. *Int J Appl Pharm*, 2017;9(6):60.
37. Gandhi AK, Kang J, Capone L, Parton A, Wu L, Zhang LH, *et al.* Dexamethasone synergizes with lenalidomide and pomalidomide to inhibit multiple myeloma tumor growth by targeting the tumor microenvironment. J Hematol Oncol, 2014;7:64.
38. Richardson PG, Siegel DS, Vij R, Hofmeister CC, Baz R, Jagannath S, *et al.* Pomalidomide alone or in combination with low-dose dexamethasone in relapsed and refractory multiple myeloma: a randomized phase 2 study. Blood, 2014;123(12):1826-32.
39. Jacob S, Kather FS, Boddu SHS, Attimarad M, Nair AB. Nanosuspension innovations: expanding horizons in drug delivery techniques. *Pharmaceutics*, 2025;17(1):136.

Cite this article: Thabbssum SS, Bhikshapathi DVRN, Jaini PK, Viswaja M, Pasha4 NDA. Design of Experiments (DoE)-Based Optimization of Pomalidomide Nanosuspension for Improved Oral Absorption and Pharmacokinetics. Indian J of Pharmaceutical Education and Research. 2026;60(3s):s1024-s1036.

Supplementary Table 1: Selection and justification of various targeted profiles along with quality attributes.

QTPP	Target	Justification
Preparation	NS	Selected technique helps to increase the solubility and rate of dissolution.
Route of administration	p.o	To match the available dosage form
Rate of drug release	Better when compared to plain drug	When solubility improves, it will increase the rate of drug release
Kinetics	Superior when compared to drug dispersion	High Bioavailability
Stability	Up to ninety days, NS should not show a rapid rise in PS	Because PS, marks formulation's efficiency, so it is important
CQAs		
CQA	Target	Justification
PS	In Nano-range	Reducing the size to the nanolevel enhances the surface area
ZP	± 25mV	If the value is near to the stated the system will be highly stable

Supplementary Table 2: Data of variables w.r.t factors F value and R^2.

Factors	F-value (model)	Lack of fit	R^2	Adjusted R^2	Predicted R^2	Adeq Precision
PS	34.80	0.01	0.9783	0.9503	0.7217	21.55
PdI	6.46	0.51	0.5986	0.5059	0.4014	7.1788
ZP	61.49	0.72	0. 9342	0.9190	0.9012	24.0984