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Engineering Cefpodoxime Prodrug using Nanosuspension Approach to Modulate Solubility, Antimicrobial and Pharmacokinetic Profile

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Abstract

Cefpodoxime proxetil (CP) is broad-spectrum antibiotic belongs to third-generation cephalosporin family. Its low solubility and bioavailability have been a challenge for drug delivery. Nanosuspension (NS) technology has been explored in drug delivery to address the issues of drugs with poor water solubility. The study focused on developing a CP nanosuspension (CP-NS) formulation using solvent-antisolvent precipitation technique. The CP-NS was synthesized by precipitation using 0.5 % w/v sodium lauryl sulphate and 1.5 % w/v poloxamer-188 under controlled ultrasonication. CP-NS was characterized for Fourier transform infrared (FTIR), Transmission electron microscopy (TEM), Differential scanning calorimetry (DSC), and X-ray diffraction (XRD). In vitro dissolution studies revealed that CP-NS exhibit increased dissolution rate 2-folds than pure drug and 1.3-folds higher than marketed formulation. In vivo pharmacokinetic studies revealed 4.3-fold improvement in oral bioavailability of CP-NS than pure drug and marketed formulation. In conclusion, the formulation of cefpodoxime proxetil nanosuspension showed promising results in terms of drug dissolution and antimicrobial activity for prodrug based active moieties.

Keywords: Cefpodoxime proxetil, Nanosuspension, Prodrug, Solubility enhancement, Oral bioavailability enhancement, Pharmacokinetic study.

Introduction

Antibiotics are crucial for prophylactic and therapeutic treatment of bacterial infections, and supporting complex medical procedures by effectively targeting and controlling bacteria. They have contributed to more than 70% reduction in fatalities from infectious disease (1). Cephalosporin are crucial antibiotics known for their broad-spectrum activity, making them efficient at combating diverse bacterial infections (2). They are widely used due to their efficacy, lower resistance rate and low toxicity compared to other antibiotic classes (3). Cefpodoxime proxetil (CP) belongs to third-generation cephalosporin family and a broad-spectrum antibiotic. They effectively target a wide range of gram-positive and gram-negative bacteria by inhibiting the synthesis of bacterial cell wall. They effectively treat urinary and respiratory tract infections. Cefpodoxime proxetil after oral administration absorbed through the GI tract. It undergoes hydrolysis to convert into active form cefpodoxime (4). Despite being formulated to enhance permeability and bioavailability, CP still has 50% oral bioavailability (5). It is due to poor solubility in an aqueous base, which results in CP with poor dissolution characteristic leads to lower bioavailability (6). CP shows pH-dependent solubility, showing typical gelation behavior in acidic conditions. To improve its solubility various conventional and novel drug delivery approaches were explored such as sol-

id dispersion (7), solid-lipid nanoparticles (SLN) (8), self-nanoemulsifying drug delivery system (SNEDDS) (9), nanoparticles (10), nano-emulsion (11), microparticles (12).

Nanosuspension (NS) technology has great potential in drug delivery to address solubility-related challenges of drugs with poor solubility (13). A reduction in drug particle size to the nanometer range enlarges surface area for dissolution, which improves bioavailability of drug (14). Nanosuspension are colloidal dispersion of drug particles at nano-scale in an aqueous base, stabilized by suitable stabilizers (surfactant, polymers, or mixture of both). Nanosuspension are unique owing to their simplicity and advantages over other formulation strategies (15). Nanosuspension are effective in controlled drug delivery systems, maintaining the drug in crystalline state with reduced particle size, that improves bioavailability. The nanosized particles enhance saturation solubility (16). Compared to liposome and other conventional colloidal drug carriers, nanosuspension are simple, cost-effective approach to produce a physically more stable product.

Two methods employed for manufacturing nanosuspension; Bottom-up process and top-down process (17). Solvent-antisolvent precipitation method has been employed to produce submicron sized particles for drugs with poor solubility. This is easy, scalable and cost-effective technique. The drug is dissolved in organic solvent and mix with miscible antisolvent (18). This creates a high concentration gradient, which enhances supersaturation by reducing the diffusional pathway around drug nanoparticles. The disruption of drug crystal into nanoparticles can generate high-energy surfaces, increasing saturation solubility, dissolution rate, and oral bioavailability (19). The increased surface area also increases saturation solubility for nanosized drug particles (5). To ensure a stable nanosuspension and prevent Ostwald ripening, a phenomenon where smaller particles dissolve and accumulate on larger ones, it is crucial to maintain uniform particle size. This uniformity avoids instability caused by large variations in particle size (20).

The present research focused on developing CP-NS to improve the solubility and bioavailability. The Box-Behnken design with three-level, three factor was employed to formulate CP-NS by solvent-antisolvent precipitation method using probe sonication technique. Further, CP-NS were analysed for particle size, zeta potential, polydispersity index (PDI), antimicrobial study, in vitro drug release, in vivo pharmacokinetic and stability study.

Materials and Methods

Chemicals

Cefpodoxime proxetil was received as gift sample from Indoco remedies, Mumbai India. Sodium lauryl sulphate (SLS), poloxamer-188 (P-188), polyvinylpyrrolidone (PVP) K30 were sourced through Loba (Mumbai, India). O-phosphoric acid (HPLC grade), acetonitrile (ACN), methanol (HPLC grade), potassium dihydrogen phosphate, sodium dihydrogen orthophosphate anhydrous, were purchased from Merck and Loba (Mumbai, India).

Preliminary studies

Selection of stabilizer - Stabilizer were screened among categories like: Anionic stabilizers (SLS), Non-ionic stabilizers (PVA, Tween 80, PEG, P-188), and Polymeric stabilizers (HPMC, PVP K30) (21). The CP is cationic in nature to provide better stabilization the anionic and non-ionic stabilizers were selected (22). The solubility of CP in the various stabilizer solutions was evaluated using incubator shaker. 1% w/v stabilizer solution (10 ml) prepared and excess amount of drug was added. These sample were shaken on an incubator shaker at the maximum speed of 20 rpm for 24 h to reach equilibrium (23). After 24 h, sample were filtered through Whatman filter paper. The aliquot was analysed by UV-spectrophotometer at 235 nm.

QbD approach for optimization of CP nano-suspension

Experimental design

The conventional approaches for drug formulation development often face challenges being time consuming, unpredictable and costly. To address these pitfalls Design of Experiment (DoE) was utilized. DoE provides systematic way to investigate the effect of variables on responses. It reduces costs and time while ensuring reliable, reproducible optimization and more accurate identification of optimal conditions (24). The Box-Behnken Design (BBD) provides several benefits over traditional methods, such as more efficient resources use and simpler experimentation (25). It requires fewer runs for accurate results, enabling precise modelling of interactions and quadratic effects. The BBD with three variables and three level were applied to optimize the CP-NS. In this design, the experimental space was considered as cube (26). As per BBD, 15 runs were conducted with three variables and three level. The mid-point was repeated three times. On the basis of preliminary analysis the experimental range of variables selected for design. The independent variables (X) are concentration of (stabilizer) P-188 (X_1), concentration of (surfactant) SLS (X_2), and ultrasonication time (X_3) with three levels (−1, 0, and +1). The dependent variables (Y) are particle size (Y_1) and percent entrapment efficiency (Y_2). These variables were chosen due to their significant effect on responses. Table 1 and 2 display the variable design and data of all 15 runs. The design and assessment of experiment were conducted using Design-Expert® software version 11.

Table 1. Variables and levels in Box-Behnken design

Factors	Levels		
Independent variables	Low	Medium	High
X□= Concentration of poloxamer-188 (mg)	10	30	50
X□= Concentration of SLS (mg)	5	10	15
X_3= Ultrasonication time (min)	5	10	15
Response variables			
R□: Particle size (nm)	Minimize		
R□: Entrapment efficiency (%)	Maximize		

Table 2. Box-Behnken design experimental runs.

Batch	X_1 (P-188: mg)	X_2 (SLS: mg)	X_3 (Time: min)
1	50	5	10
2	50	10	5
3	50	10	15
4	50	15	10
5	30	5	5
6	30	5	15
7	30	10	10
8	30	10	10
9	30	10	10
10	30	15	5
11	30	15	15
12	10	5	10
13	10	10	5
14	10	10	15
15	10	15	10

$$Yi = b0 + b1X_1 + b2X_2 + b3X_2 + b1X_1^2 + b2X_2^2 + b3X_3^2 \quad \text{……. (1) (26)}$$

The response for each factor at particular level predicted by the regression equation expressed as coded variables. The relative impact of the variable can be determined by coded equation.

Preparation of nanosuspension

Cefpodoxime proxetil nanosuspension (CP-NS) was formulated by the solvent-antisolvent precipitation technique. Weighed quantity of CP was dissolved in ethanol. Simultaneously to prepare an aqueous solution the required quantity of P-188 as a stabilizer and SLS as surfactant was dispersed in distilled water. Ethanol-CP solution were dropwise added into aqueous base at 1200 rpm under magnetic stirring. This dispersion was further subjected to probe ultrasonication for 10 min with 30% amplitude as represented in Figure 1 (27).

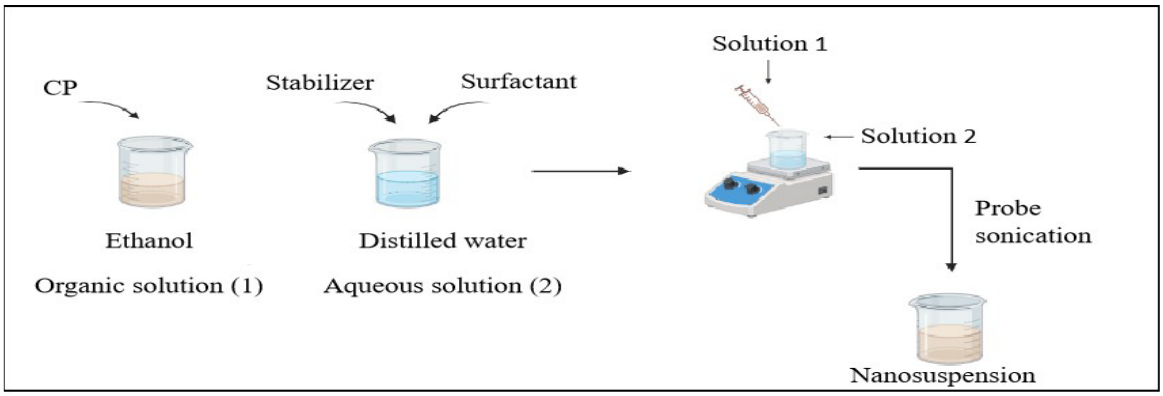

Figure 1. Preparation of cefpodoxime proxetil nanosuspension (CP-NS) by solvent-antisolvent precipitation method.

Analytical characterization of nanosuspension

Particle size and zeta potential

The particle size and zeta potential of CP-NS were analyzed by particle size analyzer (Nanopartica, HORIBA Scientific). All experiments were conducted in triplicates.

Entrapment efficiency

To determine entrapment efficiency (% EE), the free drug present in 1mL CP-NS dispersion was determined by centrifugation (Allegra 64R Benchtop Centrifuge, Beckman Coulter, USA) (28). 1mL of prepared formulation was centrifuged at 18,000 for 30 minutes. The supernatant is separated, filtered and analyzed by UV-spectrophotometer (JASCO V- 730, Japan) at 235 nm. The study was performed in triplicate. The entrapment efficiency was determined using the following equation:

$$\%EE = \frac{Total\ amount\ of\ drug\ added - amount\ of\ free\ drug}{Total\ amount\ of\ drug\ added} \times 100$$

Transmission electron microscopy

The morphological evaluation of CP-NS was analyzed by Transmission electron microscopy (TEM, Philips, CM 200). A thin layer of CP-NS placed on copper grid (#400) and negatively stained by phosphotungstic acid. The solution was applied to grid and allowed to sit for 60 seconds. At ambient temperature, the TEM images were captured (29).

FTIR analysis

FTIR spectra of CP, physical mixture (PM) and CP-NS were obtained with JASCO FTIR-4100 spectrometer (Japan). Potassium Bromide (KBr) was combined with sample (2-3mg) and filled in the cavity. The sample was scanned over the range of 4000 - 400 cm^{-1} wave numbers (30).

Saturation solubility

Solubility studies of CP and CP-NS formulation were evaluated by adding excess drug and lyophilized CP nanosuspension to the vial

containing water, hydrochloride buffer (0.1N HCL) and phosphate buffer (PBS). The vials were placed on shaker incubator for about 24h, then a specific volume of aliquots was taken and centrifuged at 15,000 rpm at 25ºC for 15 minutes (23).The supernatant was filtered and assayed using UV-visible spectrophotometer (JASCO V-730, Japan) at 235 nm. The results measured in triplicate.

Differential scanning calorimetry studies (DSC)

DSC was carried out using a thermal analysis system (DSC7020, HITACHI) of CP, P-188, SLS, PM, and CP-NS. The 1mg sample were heated at 1- 400°C at a constant rate of 10°C/min, in aluminium pan under a nitrogen atmosphere (31).

X-ray diffraction studies (XRD)

The XRD analysis of CP, PM and CP-NS were recorded on X-ray diffractometer (Miniflex 300/600, Rigaku Tokyo, Japan). The samples were kept in aluminium sample holder and scanning rate was about 1°/min and the scanning range of 2θ in the range of 2-80° (31).

In-Vitro dissolution profile

Dialysis bag technique was employed to determine the drug release profile of pure drug (CP dispersion), CP-NS formulation, and CP marketed formulation (suspension). The dialysis bag (molecular weight:12000 -14000 g/mol) was prepared by soaking it in dissolution media for 24 h before initiation of study. A pre-treated dialysis bag was filled with 2ml of CP-NS and sealed at both ends. The sac was placed in 40 ml of freshly prepared acidic medium (pH 1.2) for 4 h with stirring at 100 rpm on magnetic stirrer (REMI 1MLH). 1ml of sample were drawn at pre-determined time points (0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, and 5h) and same volume of fresh media added to maintain sink condition. The release study of CP dispersion and marketed formulation were conducted with same experimental conditions. The samples were filtered and analyzed using UV-spectrophotometer at 235 nm. All experiment conducted in triplicate and data were analyzed using kinetic models (zero-order, first-order, korsemeyer-peppas, and Higuchi). The mechanism of drug release were determined by using the regression coefficient (r^2). Invitro release data of CP-NS was compared with pure drug and marketed formulations (32).

Antimicrobial efficacy study

The assessment of antimicrobial activity was done by agar well diffusion test. The medium used was Mueller Hinton agar for *E.coli* and *S.aureus*. Micro-organisms were inoculated into Mueller Hinton agar medium, and kept at 45ºC. A microbial suspension (100 µl) was spread uniformly on surface of Mueller Hinton agar for inoculation. Then, four perforation each of depth 5 mm were made in which the marketed formulation, CP, CP-NS and dimethyl sulfoxide (control) were placed. Petri-plates were incubated at 37ºC for 24 h. The size of inhibition zone (mm) around micro-organism was used to determine the results (33).

In-vivo pharmacokinetic study

Animal and dosing

Male wistar rats of 200-250 g were procured from Global Biosciences Pvt Ltd. The Institutional Ethics Animal Committee (IEAC) approved the investigation and experiment were conducted in compliance with CPCSEA guideline in India. Re. No. 1703/PO/Re/S/01/CPCSEA, dated 17/06/2016 Approval No. CPCSEA/PCP/2024-1-15. The rats were randomly assigned to three groups, each consisting six animals and housed under controlled conditions of 25±1ºC and humidity 55±5% with 12 h light/dark condition. Before the initiation of experiment, the animals were fasted overnight but had free access to water. Oral administration of CP, CP-NS and CP marketed formulation at dose of 10 mg/kg were received by three groups of rats (12). After oral administration 0.5 ml blood was collected from the retro-orbital plexus at

predefined intervals of 0.5, 1, 2, 4, 6, 8, 12, and 24h. Plasma sample were collected and centrifuged at 6000 rpm for 15 min at 4 ºC and stored at -20 ºC (9,34).

Plasma sample preparation and analysis

CP was determined by HPLC (Jasco,PU-2080 plus, UV-2075plus, Japan) method. The mobile phase was composed of ammonium acetate buffer (pH 5) and acetonitrile 55:45 v/v and was eluted at flow rate of 1.0 ml/min with UV detection at 235 nm. Plasma samples were precipitated with methanol in 1:1 ratio. The sample centrifuged at 6000 rpm for 10 min and filtered by 0.45µm membrane filter. The supernatant (20µL) was introduced into the HPLC for analysis (35).

Pharmacokinetic and statistical analysis

Analysis of pharmacokinetic parameter was performed by PKSolver, Microsoft Excel add-in (version 2.0). The pharmacokinetic profiles of t_{max}, C_{max} and $AUC_{0\rightarrow24h}$ were determined.

Stability studies

The stability of CP-NS formulation were investigated under two distinct condition 4ºC and 30ºC/60% RH for 3 months. Particle size and PDI analysis of formulation were performed (36).

Results and Discussion

Selection of stabilizer

Preliminary selection of stabilizer was based on drugs solubility in selected stabilizer. CP showed maximum solubility in tween 80, which could be due to amphiphilic nature of CP. If drug is more soluble in stabilizer solution, leading to Ostwald ripening. It is phenomenon in which the particle size increases on storage leading to instability of nanosuspension. Hence tween 80 were not consider for further studies (9). P-188 shown lowest solubility as shown in Figure 2. Preparation of CP-NS with P-188 results in least particle size and PDI. On the other hand, the particle size of P-188 with SLS remains unchanged compared to P-188 alone. Hence, to prevent the aggregation of nano-sized particle the combination of P-188 and SLS were preferred as steric stabilizer.

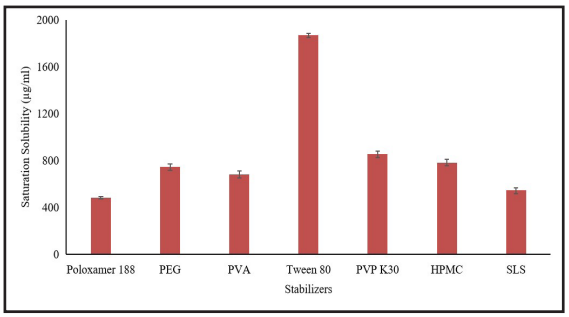

Figure 2. Saturation solubility of Cefpodoxime proxetil (CP) with different stabilizers.

Development of CP nanosuspension and experimental Design

The influence of the concentration of P-188 (X_1), concentration of SLS (X_2), and the ultrasonication time (X_3) on response particle size (Y_1) and entrapment efficiency (Y_2) were analyse by using Box-Behnken Design (BBD). Table 3 contain response information corresponding to each of 15 runs. The responses were analysed using various statistical parameters to select a quadratic model. The predicted R^2 and adjusted R^2 values ranges from 0.98 to 1, model with statistical significant p-value ($p < 0.05$) and p-value ($p > 0.10$) reflecting an insignificant lack of fit. The particle size (Y_1) and entrapment efficiency (Y_2) of the responses vary between 126±18 nm and 305±7 nm and 52.88±19.13% to 86.52±25.56%, respectively.

Table 3. BBD presenting the value of response obtained for CP nanosuspension

Run	X_1	X_2	X_3	Y_1	Y_2
	mg	Mg	min	nm	%
1	50	5	10	292 ± 4	64.47 ± 7.1
2	50	10	5	176 ± 12	52.88 ± 19.13
3	50	10	15	126 ± 18	83.26 ± 8.11
4	50	15	10	280 ± 14	68.32 ± 10.42
5	30	5	5	240 ± 18	59.65 ± 21.68
6	30	5	15	157±16	68.12 ± 9.78

7	30	10	10	152 ± 10	86.52 ± 25.56
8	30	10	10	143 ± 9	74.35 ± 13.25
9	30	10	10	148 ± 21	79.98 ± 6.47
10	30	15	5	209 ± 5	84.98 ± 12.5
11	30	15	15	176 ± 20	52.72 ± 19.44
12	10	5	10	305 ± 7	69.87 ± 16.34
13	10	10	5	230 ± 14	68.38 ± 2.15
14	10	10	15	126 ± 10	69.32 ± 8.74
15	10	15	10	298 ± 19	74.21 ± 17.14

[(X_1)-Concentration of Poloxamer-188, (X_2)-Concentration of SLS, (X_3)-Ultrasonication time, (Y_1)- Particle size and (Y_2)- Entrapment efficiency. Data are presented as mean ± SD (n = 3)]

Table 4 presents analysis of variance (ANOVA) for response variable of particle size (Y_1) and entrapment efficiency (Y_2). If p-value of model is ($p < 0.05$), ANOVA suggest response is significant. ANOVA determines the degree of influence, significance and correlation. Variance in adjusted R^2 and predicted R^2 was less than 0.3, indicating that the values of the response were precisely predicted by the model. Polynomial Eqs. (3), (4) are derived from regression analysis.

$$Y1= 152-6A-16.13B-39.88C-1.25AB+42.75AC+24BC+62.92A2+45.17B2-19.83C2 \quad .....(3)$$

$$Y2 = 85-2.23A+0.84B-2.61C-0.29AB-2.88AC-7.10BC-6.07A2-9.87B2-11.79C2 \quad ....(4)$$

In model, the negative coefficient indicates a contradictory effect, whereas a positive coefficient suggests a synergistic effect.

Table 4. Quadratic response surface model of the ANOVA for particle size and entrapment

Source	Sum of square Particle size (nm)	Entrapment efficiency (%)	F-value Particle size (nm)	Entrapment efficiency (%)	P-value Particle size (nm)	Entrapment efficiency (%)
Model	54777.52	1222.89	8.33	27.47	0.0155	0.0010
X1	1352.00	39.92	1.85	8.07	0.2320	0.0362
X2	1176.13	5.76	1.61	1.17	0.2605	0.3297
X3	780.13	54.29	1.07	10.97	0.3490	0.0212
X1X2	6.25	0.3481	0.0085	0.0704	0.9299	0.8014
X1X3	4556.25	33.24	6.23	6.72	0.0547	0.0487
X2X3	3364.00	201.50	4.60	40.73	0.0848	0.0014
$X1^2$	13255.41	135.97	18.13	27.49	0.0080	0.0033
$X2^2$	17157.03	359.94	23.47	72.76	0.0047	0.0004
$X3^2$	11101.64	512.88	15.19	103.68	0.0114	0.0002
Residual	3655.42	24.73	-	-	-	-
Lack of fit	710.75	17.62	0.1609	1.65	0.9143	0.3988
Pure error	2944.67	7.12	-	-	-	-
Cor Total	58432.93	1247.62	-	-	-	-

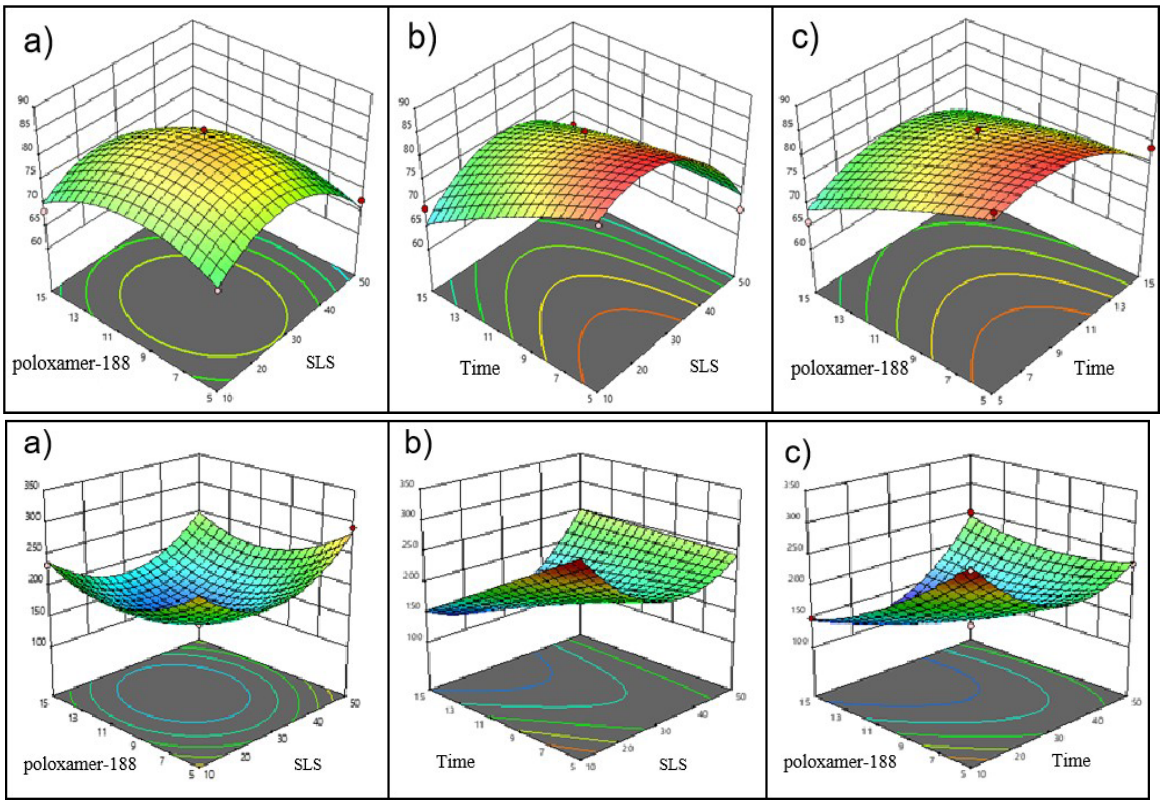

Figure 3. Response surface plot illustrating the effect of concentration of the Poloxamer-188, concentration of the Sodium lauryl sulphate (SLS) and Ultrasonication time upon 1) Particle size (nm) and 2) Entrapment efficiency (%).

Effect on particle size

Particle size is key parameter in improving the solubility of drugs with poor solubility. A decrease in particle size, increases surface area leading to improved saturation solubility. Figure 3-1. a) illustrates the impact of P-188 concentration on particle size. The optimal concentration of P-188 significantly reduces the particle size in the nanosuspension. However, both lower and higher concentrations of the stabilizer lead to increased particle size. At low stabilizer concentrations, resulting in larger particle sizes. The effect of concentration of SLS was similar to P-188. Furthermore, Figure 3-1.c) demonstrates that increasing ultrasonication time significantly reduces particle size in the nanosuspension. This reduction was attributed to the fragmentation of particles facilitated by ultrasonic waves (28).

Effect on entrapment efficiency

The concentration of P-188 significantly impacts entrapment efficiency, with both low and high concentrations. As P-188 concentration increases, flocculation and aggregate formation occur, resulting in lower entrapment efficiency (37). Conversely, increasing the concentration of SLS shows a slight improvement in entrapment efficiency, as depicted in Figure 3-2.b) Additionally, prolonged ultrasonication time gradually reduces entrapment efficiency, likely due to increased attrition and generation of mechanical energy. This extended sonication causes larger particles to break down into smaller ones, further affecting entrapment efficiency.

Establishment of design space and model validation

Design-Expert software (version 11) was used to assess design space in order to achieve optimized CP-NS. A numerical optimization approach was used to select optimized CP-NS, based on desirability value near to 1. The optimal formulation were determined by criteria of minimum particle size and maximum entrapment efficiency. The optimized nanosuspension (b7) was prepared with 0.5% w/v SLS, 1.5 % w/v P-188 under controlled sonication time. The observed R^2 and predicted R^2 values are 0.995- 0.988 shows a linear correlation (38). The results of validation trials showed robustness and feasibility of design.

TEM Analysis

The morphology of CP-NS was analysed by TEM. The resulting TEM micrograph of CP nanosuspension was presented in Figure 4. TEM analysis showed suspended nanoparticles was uniformly distributed and approximately spherical in shape. The size of the nanoparticles was found to be between 150-250 nm.

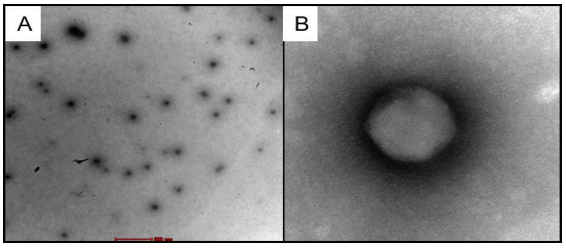

Figure 4. A. TEM image of Cefpodoxime proxetil (CP) nanosuspension on 500 nm scale and B. 100 nm scale.

FT-IR analysis

FTIR was used to evaluate any chemical modification between drug (CP) and stabilizer (P-188, SLS). The spectra of CP, P-188, SLS, PM and CP-NS were shown in Figure 5. CP displayed a characteristic amide C-N stretching vibration band at 1650 cm^{-1}, a C-O stretching band at 1074 cm^{-1} and carbonyl C-H band at 2950 cm^{-1} (39). The FTIR spectra of P-188 display aromatic C-H stretching peak at 2851 cm^{-1}, C-O-C poly (ethylene oxide) stretching at 1079 cm^{-1} and O-H stretching peak at 3404 cm^{-1}. For SLS C-O stretching at 1657 cm^{-1} and C-O stretching at 1657 cm^{-1} was observed. Consistent with the literature finding (40) Figure 5 presents the spectra for CP, P-188, and SLS. The interaction between the drug and stabilizer can be significantly influence these characteristic peaks, either by altering the intensity or causing a variation in wavenumber. The PM showed no spectral shift being simply overlay of CP, P-188, and SLS, suggesting the stabilizer and drug had no interaction.

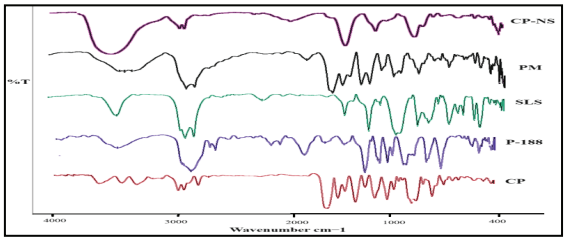

Figure 5. FTIR spectra of A. Cefpodoxime proxetil (CP) B. Poloxamer-188 (P-188) C. Sodium lauryl sulphate (SLS) D. Physical mixture (PM) E. Cefpodoxime proxetil nanosuspension (CP-NS).

Differential scanning calorimetry

DSC analysis was conducted to confirm the physical state of CP in nanosuspension and compare it with CP, P-188, SLS and PM. As shown in Figure 6, CP exhibited single melting endothermic peak at 95.1ºC, with a fusion enthalpy of (ΔH) of 0.76 mW/min. The DSC profiles of P-188 and SLS exhibited sharp melting peaks at 49.53ºC and 198.47ºC respectively. The PM exhibited broadened endothermic peak of CP at 103ºC with an enthalpy change (ΔH) at 1.65 mW/min, indicating possible interaction between drug and excipients. The presence of distinct peak suggest the drug retain some of its crystalline properties in PM (41). The appearance of same peaks between PM and CP-NS

can be attributed to melting peaks of P-188 and SLS. In the CP-NS, melting peak of CP was absent, indicates reduction in crystallinity and conversion to amorphous state. The amorphization is attributed to nanosizing process and stabilization by excipient such as P-188 and SLS during the formation of nanosuspension (42). These finding suggest that during nanosuspension formation, CP nanoparticles transitioned from a crystalline state to amorphous state which improve solubility and beneficial for enhancing the bioavailability of drug (43).

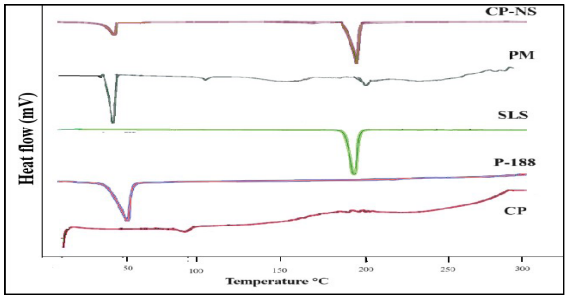

Figure 6. DSC patterns of A. Cefpodoxime proxetil (CP) B. Poloxamer-188 (P-188) C. Sodium lauryl sulphate (SLS) D. Physical mixture (PM) E. Cefpodoxime proxetil nanosuspension (CP-NS).

X-ray diffraction

XRD analysis were carried out for validation of crystalline form of CP and the amorphous state of CP nanosuspension. Figure **7** shows the XRD of CP, PM, and CP-NS. The diffraction pattern revealed that CP exhibited distinct high-energy diffraction peak at 19.88º and 23.35º, which demonstrated that CP was crystalline in nature. Due to crystalline structure, it often results in poor solubility. The diffraction pattern of the PM reveal the peak similar to CP which indicate CP in crystalline form. The CP-NS displayed peaks at 31.55º and 40.03º, with significant reduction in intensity of crystalline peaks and some of the characteristic peaks of CP was absent. This suggest that crystalline CP was transitioned to amorphous form in the nanosuspension (44). A drug in amorphous form possesses a higher-energy state, which provides the benefit of enhanced solubility. This results in a faster dissolution rate and consequently, improved oral bioavailability (45).

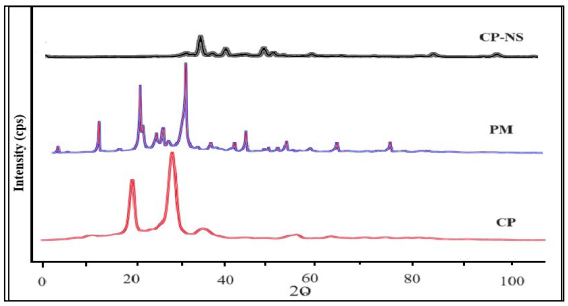

Figure 7. XRD spectrum of A. Cefpodoxime proxetil (CP) B. Physical mixture (PM) C. Cefpodoxime proxetil nanosuspension (CP-NS).

Stability studies

Physical stability study was carried out by storage of CP-NS at two different conditions at 4ºC and 30ºC/60% RH over a period of 3 months. The particle size and PDI was assessed after preparation and periodically over a 3 month under various conditions Table 5. The particle size of formulation stored at 30ºC/60% RH increased significantly ($p < 0.05$) after 3 months. In contrast, the formulation stored at 4ºC showed no significant change in particle size or PDI (38). The CP-NS formulation shown physical instability at 30ºC/60% could be due to aggregation in nanosuspension, which was influenced by high temperature and humidity. These factors are known to adversely affect drug nucleation and accelerate the rate of crystallization.

Table 5. Comparison of the mean particle size and PDI of nanosuspension after 3 months of storage at 4ºC and 30ºC with initial values.

	Initial	1 month		3 month	
		4 ºC	30 ºC	4 ºC	30 ºC
Particle size (nm)	136 ± 5	140 ± 5	150 ± 8	145 ± 10	170 ± 10
PDI	0.22 ± 0.08	0.22 ± 0.3	0.23 ± 0.3	0.22 ± 0.4	0.24 ± 0.5

Saturation solubility

CP is crystalline in nature and exhibit poor solubility. The saturation solubility in water was 0.266 ± 0.004 mg/mL. However, upon formulation into nanosuspension (CP-NS) showed five-fold increase in saturation solubility in water. It is due to significant reduction of particle size in the nanosuspension. The reduced particle size leads to larger surface area, facilitating better interaction with dissolution media (46). The saturation solubility was carried out in different pH conditions (1.2, 6.8). As shown in Figure 8, the results of saturation solubility depicts CP exhibits pH-dependent solubility. CP has ionizable functional group that change with pH levels. In acidic pH drug remain protonated thus increasing its solubility. However, at alkaline pH, deprotonation can cause degradation of drug and decrease its solubility (47).

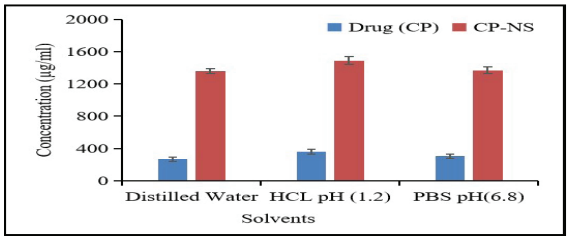

Figure 8. Saturation solubility of Cefpodoxime proxetil (CP) and Cefpodoxime proxetil nanosuspension (CP-NS) in distilled water, HCL pH (1.2) and PBS pH (6.8).

In-Vitro Dissolution Study

The dissolution profile for CP, CP-NS and CP marketed formulation were shown in Figure 9. The CP-NS exhibit better in-vitro drug release than CP and CP marketed formulation. The release of CP from the CP-NS was > 90% within 2 h, while those for pure drug was 40% and 65% for CP marketed formulation. The reason might be enhanced surface area by reducing particle size, decreases thickness of diffusional layer, and increases concentration gradient between particle surface and bulk solution, thereby improving bioavailability (46). Furthermore the dissolution rate significantly influence by state of drug in nanosuspension. Previous studies, including XRD and DSC analysis, indicated the amorphization of CP in CP-NS formulation. The drug in amorphous form has increased molecular mobility and higher internal energy promoting solubility (48). CP-NS release followed the Korsmeyer–Peppas model (R=0.97, n=0.063) indicating exponential drug release over time.

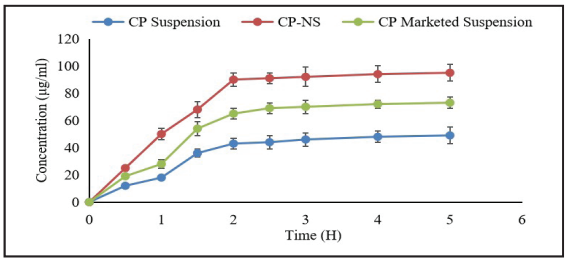

Figure 9. Comparative in-vitro dissolution profile of the Cefpodoxime proxetil (CP), Cefpodoxime proxetil nanosuspension (CP-NS) and CP marketed formulation.

Antimicrobial Efficacy Study

The antimicrobial activity of CP were investigated by using *E. coli* and *S. aureus* micro-organism. For 24 h, the petri plates were incubated at 37ºC. The results were assessed by measuring diameter (mm) of the inhibition halo around microbial growth and the calculation of the area of inhibition (49). The antimicrobial activity of the CP-NS against the *E. coli* and *S. aureus* micro-organism can be depicted with the help of petri plate photograph. Figure 10 (1) and 10 (2) depicts the CP-NS showed 36.5 mm^2 and 32.6 mm^2 area of inhibition compared with CP marketed formulation, pure CP and DMSO against *E. coli* and *S. aureus* micro-organism, respectively.

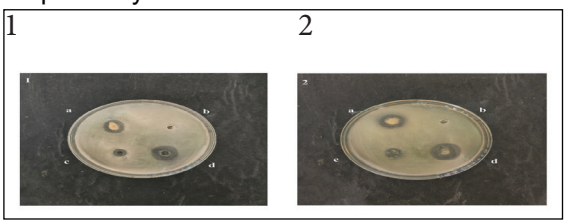

Figure 10. Average inhibition halo (mm) against 1. *Escherichia coli*, 2. *Staphylococcus aureus.*

In-vivo pharmacokinetic study

The plasma concentration of CP was measured by a HPLC method, which demonstrated linearity within the range of 1-1000 µg/mL and exhibited high accuracy and specificity. The Table 6 present pharmacokinetic data for orally administered CP, CP marketed formulation and CP-NS. The plasma drug concentration-time profiles after oral administration at a dose of 10 mg/kg CP solution shown in Figure 11.

Table 6. Pharmacokinetic parameters of CP in wistar rats after oral administration of CP, CP marketed formulation, and CP-NS.

Parameters	CP	CP marketed formulation	CP-NS
C_{max} (µg/mL)	355±20	621±28	1624±214
T_{max} (h)	2	2	2
AUC $_{0\rightarrow24h}$ (µg/mL)	1542±34	2674±54	7117±320

*Data are presented as mean ± SD (n = 3).

Pharmacokinetic data reveals significant and remarkable insights. A significant difference was observed in the plasma concentration-time profile of CP, CP-NS, and CP marketed formulation (Figure 11). C_{max} and AUC are key parameters revealing insights about the rate and extent of absorption. The results shows 4.5-folds and 2.6-folds higher C_{max} of CP-NS when compared to C_{max} of CP and CP marketed formulation respectively. The relative bioavailability of CP-NS was 4.3-folds as compared to CP and CP marketed formulation. In-vivo results of current study were better than the literature which reports o/w submicron emulsion of CP with 2.1-folds enhanced AUC of optimized batch (50). The outcome of in-vivo study showed a remarkable enhancement in the absolute bioavailability of CP as nanosuspension. This was because the decrease in particle size leads to increase in rate of dissolution as explained by Noyes-Whitney equation.

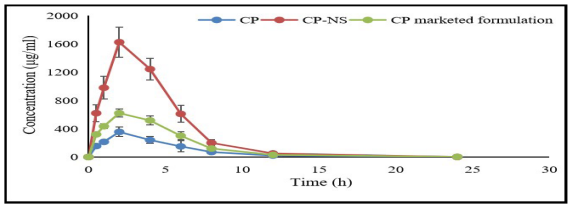

Figure 11. Plasma drug concentration time profile of the Cefpodoxime proxetil (CP), Cefpodoxime proxetil nanosuspension (CP-NS) and CP marketed formulation after oral administration (10 mg/kg).

Conclusion

Cefpodoxime proxetil nanosuspension using the solvent-antisolvent precipitation technique is successfully prepared to enhance solubility and antimicrobial activity. Optimization using the Box-Behnken Design resulted in batch (B7), which shows a low particle size and highest entrapment efficiency. The particle size of developed nanosuspension ranged from 126 ± 18 to 305 ± 7 nm. Physicochemical characterisation, such as DSC and XRD studies confirmed the formation of amorphous CP nanoparticles within the nanosuspension. The CP-NS showed a 5-fold enhanced solubility. In vitro dissolution studies revealed that CP-NS exhibit increased dissolution rate 2-fold than pure drug and 1.3-fold higher than marketed formulation. Stability studies indicated that nanosuspension was stable when stored at 4ºC. The storage conditions affected particle size and PDI. Antimicrobial efficacy studies demonstrated the effectiveness of CP-NS against *E.coli* and *S.aureus* micro-organism. Finally, in vivo pharmacokinetic data showed 4.3-fold improvement in bioavailability of CP-NS than pure drug and marketed formulation. Overall, CP-NS formulation showed promising results in terms of solubility enhancement and antimicrobial activity. Further studies, the pharmacodynamic study can be performed to confirm the safety and efficacy of the cefpodoxime proxetil nanosuspension. Studies on long-term stability will ensure the product's quality

and shelf life, which is necessary for a successful commercialization.

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Author Contribution

Prerana Bhosale wrote original draft, methodology, validation, investigation. Dr. Vividha Dhapte-Pawar conceptualized, investigated, data curated. Priyanka Gawarkar-Patil reviewed and edited. Dr. Atmaram Pawar supervised and critiqued the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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