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FORMULATION, CHARACTERIZATION AND EVALUATION OF CEFIXIME TRIHYDRATE NANOEMULSION

Mr. Ganesh V. Shinde[1*], Dr Jadhao U.T[2], Mr. Rathod D.A.[3], Dr. G.N Dhembare[4]

[1*]M.Pharm Scholar, SVP College of Pharmacy, Hatta, Tq. Basmat, Dist, Hingoli, Maharashtra- 431705

[2]Principal, SVP College of Pharmacy, Hatta, Tq. Basmat, Dist, Hingoli, Maharashtra- 431705

[3]Assistant Professor, SVP College of Pharmacy, Hatta, Tq. Basmat, Dist, Hingoli, Maharashtra- 431705

[4]Associate Professor, SVP College of Pharmacy, Hatta, Tq. Basmat, Dist, Hingoli, Maharashtra- 431705

Abstract:

Background: *Cefixime Trihydrate is a third-generation cephalosporin antibiotic with broad-spectrum antibacterial activity; however, its clinical efficacy is limited by poor aqueous solubility and low oral bioavailability. The present study aimed to develop and evaluate a nanoemulsion formulation to enhance the solubility, dissolution, and oral delivery of Cefixime Trihydrate.*

Methods: *Preformulation studies, including physicochemical characterization, melting point determination, solubility analysis, FTIR, DSC, UV-visible spectrophotometry, and HPLC, were performed. Solubility screening was conducted to select suitable formulation components. Mentha oil, Tween 80, and PEG 200 were selected as the oil phase, surfactant, and co-surfactant, respectively. Pseudo-ternary phase diagrams were constructed to identify the nanoemulsion region, and formulations were optimized based on Smix ratio, Oil:Smix ratio, and physicochemical stability. The optimized nanoemulsion was evaluated for pH, viscosity, density, refractive index, self-emulsification time, drug content, particle size, zeta potential, thermodynamic stability, in vitro drug release, and accelerated stability according to ICH guidelines.*

Results: *FTIR and DSC analyses confirmed the compatibility of Cefixime Trihydrate with the selected excipients. The optimized formulation (Batch A7) exhibited satisfactory physicochemical properties with a pH of 7.30, viscosity of 1.25 cP, drug content of 99.62 ± 0.147%, particle size of 132.8 nm, and zeta potential of −32.3 mV, indicating excellent colloidal stability. The formulation demonstrated rapid self-emulsification, high transmittance, and good thermodynamic stability. In vitro dissolution studies showed 98.94% cumulative drug release within 60 min, compared with 58.65% for the pure drug. Accelerated stability studies confirmed that the optimized nanoemulsion remained physically and chemically stable for 90 days without significant changes in drug content or appearance.*

Conclusion: *The developed Cefixime Trihydrate nanoemulsion significantly improved the solubility, dissolution rate, and physicochemical stability of the drug. The optimized formulation exhibited excellent drug loading, nanosized droplets, rapid drug release, and satisfactory stability, indicating its potential as an effective oral drug delivery system for enhancing the bioavailability of Cefixime Trihydrate. Further in vivo pharmacokinetic and pharmacodynamic studies are warranted to establish its clinical applicability.*

Keywords:

Cefixime Trihydrate, Nanoemulsion, Oral Drug Delivery, Solubility Enhancement, Tween 80, Mentha Oil, PEG 200, Pseudo-ternary Phase Diagram, In Vitro Drug Release.

Corresponding author:
Ganesh V. Shinde,
M.Pharm Scholar,
SVP College of Pharmacy, Hatta,
Tq. Basmat, Dist, Hingoli, Maharashtra- 431705
Email: ganeshshinde2093@gmail.com

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INTRODUCTION:

Cefixime Trihydrate is a third-generation cephalosporin antibiotic widely prescribed for the treatment of respiratory tract infections, urinary tract infections, otitis media, gonorrhea, and other bacterial infections caused by susceptible Gram-positive and Gram-negative microorganisms.[1-3] It exerts its antibacterial activity by inhibiting bacterial cell wall synthesis through binding to penicillin-binding proteins, resulting in bacterial cell lysis and death. Despite its broad-spectrum antimicrobial activity and favorable safety profile, the clinical effectiveness of Cefixime Trihydrate is compromised by its poor aqueous solubility, limited gastrointestinal dissolution, and relatively low oral bioavailability (approximately 40–50%). These limitations often result in delayed onset of action, variable therapeutic response, and the need for higher or repeated doses to maintain adequate plasma drug concentrations.[4-5]

Improving the oral delivery of poorly water-soluble drugs remains a significant challenge in pharmaceutical formulation. Various formulation strategies, including solid dispersions, cyclodextrin inclusion complexes, lipid-based formulations, nanoparticles, and self-emulsifying drug delivery systems, have been investigated to overcome solubility-related limitations. Among these approaches, nanoemulsion technology has gained considerable attention because of its ability to enhance the solubility, dissolution rate, stability, and oral bioavailability of lipophilic and poorly soluble drug molecules. Nanoemulsions are thermodynamically or kinetically stable colloidal dispersions composed of oil, surfactant, co-surfactant, and an aqueous phase, with droplet sizes generally ranging from 20 to 200 nm. The extremely small droplet size provides a large interfacial surface area, facilitating rapid drug dissolution and improved absorption across the gastrointestinal membrane. Furthermore, nanoemulsions improve drug wettability, protect the incorporated drug from degradation, reduce interfacial tension, and promote lymphatic transport, thereby minimizing first-pass metabolism and enhancing systemic bioavailability. Their excellent physical stability, ease of preparation, and scalability has made nanoemulsions an attractive platform for oral drug delivery.[6-7]

The selection of appropriate formulation components plays a critical role in the successful development of nanoemulsions. The oil phase determines the drug-loading capacity, while surfactants and co-surfactants reduce interfacial tension and stabilize nanosized droplets. In the present investigation, Mentha oil was selected as the oil phase because of its superior solubilizing capacity for Cefixime Trihydrate. Tween 80, a non-ionic surfactant with a high hydrophilic-lipophilic balance (HLB), was employed to facilitate spontaneous emulsification, whereas polyethylene glycol 200 (PEG 200) served as the co-surfactant to improve interfacial flexibility and enhance nanoemulsion stability. The formulation was optimized using pseudo-ternary phase diagram studies to identify the composition capable of producing a stable and transparent nanoemulsion. Comprehensive characterization of nanoemulsion formulations is essential to ensure their quality, stability, and performance. Parameters such as droplet size, zeta potential, viscosity, pH, refractive index, drug content, self-emulsification efficiency, thermodynamic stability, and in vitro drug release provide valuable information regarding formulation behavior and its potential for oral drug delivery. Optimization of these characteristics is directly associated with enhanced drug dissolution, improved gastrointestinal absorption, and better therapeutic efficacy.[8-10]

Therefore, the present study aimed to develop, optimize, characterize, and evaluate a nanoemulsion formulation of Cefixime Trihydrate to improve its solubility and dissolution characteristics. The optimized formulation was systematically investigated for physicochemical properties, drug–excipient compatibility, thermodynamic stability, particle size, zeta potential, in vitro drug release, and accelerated stability. The developed nanoemulsion is expected to provide an efficient lipid-based oral delivery system with the potential to enhance the

bioavailability and therapeutic performance of Cefixime Trihydrate while offering a promising alternative to conventional oral dosage forms.

MATERIALS AND METHODS:

MATERIALS:

All chemicals, reagents, solvents, and excipients used during the formulation and evaluation of the cefixime trihydrate nanoemulsion were of analytical reagent (AR), HPLC, or pharmaceutical grade to ensure the reliability and reproducibility of the experimental results. Cefixime trihydrate was obtained as a gift sample from Balaji Drugs Pvt. Ltd., Surat. Mentha oil, Tween 80 (Polysorbate 80), Castor oil, and Polyethylene Glycol 200 (PEG 200) were procured from Loba Chemie Pvt. Ltd., Mumbai. All chemicals were used as received without further purification, and freshly prepared distilled water was employed throughout the experimental work.

METHODS:

Preformulation Studies

Preformulation studies of Cefixime Trihydrate were carried out to evaluate its physicochemical properties and suitability for nanoemulsion formulation. The drug was characterized for its physical appearance (colour, odour, and texture) by visual inspection, while the melting point was determined using the standard open capillary tube method to confirm its identity and purity. Drug–excipient compatibility was assessed during the formulation development process.[11-16]

Analytical Method

The analytical method for Cefixime Trihydrate was developed using UV-visible spectrophotometry. A standard stock solution (1000 µg/mL) was prepared by dissolving 10 mg of Cefixime Trihydrate in 10 mL of ethanol. Appropriate dilutions were scanned in the wavelength range of 200–400 nm using a Jasco V-630 UV-visible spectrophotometer to determine the maximum absorption wavelength (λmax). A calibration curve was constructed by measuring the absorbance of standard solutions (2–10 µg/mL) at the determined λmax, and linearity was established using the regression equation and correlation coefficient (R^2). This validated method was subsequently employed for the quantitative estimation of Cefixime Trihydrate in the developed nanoemulsion formulations.[17-20]

Drug Identification

The identity of Cefixime Trihydrate was confirmed by Fourier Transform Infrared (FTIR) spectroscopy and UV-Visible spectrophotometry. FTIR spectra were recorded using a Shimadzu FTIR-8400S (Japan) in the range of 4000–400 cm^{-1} employing the KBr pellet method, and the characteristic absorption peaks were compared with reported reference spectra. UV-Visible spectrophotometric analysis was performed using a Shimadzu UV-1800 by scanning standard drug solutions in methanol over the wavelength range of 200–350 nm. The maximum absorbance (λmax) was observed at 287 nm, and a calibration curve was constructed using standard solutions in the concentration range of 2–14 µg/mL for quantitative analysis.[21-24]

Assay of Cefixime Trihydrate

The assay of Cefixime Trihydrate was carried out using a validated High-Performance Liquid Chromatography (HPLC) method. The chromatographic analysis was performed on a Shimadzu LC-20AD HPLC system using a mobile phase consisting of tetrabutylammonium hydroxide solution and acetonitrile (75:25, v/v) at a flow rate of 1.5 mL/min with the column maintained at 40°C. Standard and sample solutions were prepared in pH 7.0 phosphate buffer at a concentration of 0.2 mg/mL, and 10 µL of each solution was injected into the HPLC system. The assay was determined by comparing the peak area of the sample with that of the reference standard.[25-27]

Drug–Excipient Compatibility Studies

Drug–excipient compatibility was evaluated using Fourier Transform Infrared (FTIR) spectroscopy and Differential Scanning Calorimetry (DSC). FTIR spectra of pure Cefixime Trihydrate, individual excipients, and their physical mixtures (1:1, w/w) were recorded using a Shimadzu FTIR-8400S over the range of 4000–400 cm^{-1} by the KBr pellet method. The spectra were examined for any significant changes in the characteristic functional group peaks to assess potential interactions. DSC thermograms of the pure drug, excipients, and their physical mixtures were obtained using a Shimadzu DSC-60 by heating samples from 40 to 340°C at a rate of 10°C/min under a nitrogen atmosphere. The thermograms were analyzed to detect any changes in melting behavior or thermal transitions, indicating possible incompatibility.[28-30]

Solubility Studies

The solubility of Cefixime Trihydrate was determined in various oils, surfactants, and co-surfactants to identify suitable components for nanoemulsion formulation. An excess amount of drug was added to 2 mL of each vehicle and shaken for 48 h at room temperature to attain equilibrium. The mixtures were centrifuged at 5000 rpm for 15 min, filtered through a 0.45 µm membrane filter, suitably diluted, and analyzed by UV-visible spectrophotometry. Based on the highest drug solubility, Mentha oil was selected as the oil phase, Tween 80 as the surfactant, and PEG 200 as the co-surfactant for the preparation of the nanoemulsion.[31-32]

Selection of Nanoemulsion Components
The oil, surfactant, and co-surfactant were selected based on their solubilizing capacity for Cefixime Trihydrate and emulsification efficiency. Various oils, surfactants, and co-surfactants were screened through solubility studies. Mentha oil was selected as the oil phase due to its highest drug solubility, while Tween 80 and PEG 200 were selected as the surfactant and co-surfactant, respectively, based on their excellent emulsification properties and ability to produce stable nanoemulsion systems.[33-35]

Construction of Pseudo-Ternary Phase Diagram
Pseudo-ternary phase diagrams were constructed using the aqueous titration method to determine the nanoemulsion region. The surfactant (Tween 80) and co-surfactant (PEG 200) were mixed at different Smix ratios (1:1, 1:2, and 2:1, w/w) and blended with Mentha oil in varying proportions. Each mixture was titrated dropwise with distilled water under continuous magnetic stirring until turbidity or phase separation was observed. The systems were visually evaluated for clarity, transparency, flowability, and phase separation. Based on these observations, the nanoemulsion region was identified and represented using pseudo-ternary phase diagrams, which were subsequently used to select suitable component ratios for formulation development.[36-40]

Preparation of Cefixime Trihydrate Nanoemulsion
The nanoemulsion was prepared by the spontaneous emulsification method. Based on the results of the solubility studies and pseudo-ternary phase diagram, the required quantities of Mentha oil, Tween 80, and PEG 200 were accurately weighed and mixed under magnetic stirring to obtain a homogeneous isotropic mixture. Cefixime Trihydrate was dissolved in the oil phase, followed by the addition of the surfactant and co-surfactant mixture (Smix). Distilled water was then added dropwise under continuous stirring to form the nanoemulsion, which was further subjected to ultrasonication to reduce droplet size and obtain a uniform dispersion.[41-43]

Optimization of Nanoemulsion Formulation
Optimization of the nanoemulsion was performed by varying the surfactant-to-co-surfactant (Smix) ratio and the oil-to-Smix ratio. Different Smix ratios (1:1, 2:1, and 1:2) were initially evaluated for their emulsification efficiency and ability to produce transparent nanoemulsions. The optimized Smix ratio was subsequently combined with varying oil:Smix ratios (1:9 to 9:1) to identify the composition that produced a clear, homogeneous, and stable nanoemulsion. Finally, 500 mg of Cefixime Trihydrate was incorporated into the optimized formulations, which were evaluated for visual appearance, homogeneity, and drug incorporation. The formulation exhibiting maximum clarity, absence of phase separation, and satisfactory drug loading was selected for further physicochemical characterization and in vitro evaluation.[44-47]

Formulation of Cefixime Trihydrate Nanoemulsion
Cefixime Trihydrate nanoemulsions were prepared by the spontaneous emulsification method using the optimized oil, surfactant, and co-surfactant system. Mentha oil was used as the oil phase, while Tween 80 and PEG 200 served as the surfactant and co-surfactant, respectively. Initially, the drug was dissolved in the oil phase, followed by the addition of the surfactant/co-surfactant mixture (Smix). Different Smix ratios (1:1 and 2:1) and varying oil-to-Smix proportions were investigated to optimize the formulation. Distilled water was added dropwise under continuous magnetic stirring to obtain a transparent nanoemulsion, followed by ultrasonication to reduce droplet size and achieve uniform dispersion.
The prepared formulations were visually evaluated for clarity, transparency, homogeneity, and phase separation. Formulations exhibiting a clear appearance without phase separation were considered stable nanoemulsions and were selected for further physicochemical characterization and in vitro evaluation.[48-50]

Evaluation of Cefixime Trihydrate Nanoemulsion
The prepared nanoemulsion formulations were evaluated for their physicochemical properties, stability, drug content, and in vitro performance using standard analytical methods.[51-55]

Physicochemical Characterization
The pH of the formulations was measured using a calibrated digital pH meter at room temperature. Density was determined by the specific gravity bottle method, while viscosity was measured using a Brookfield digital viscometer at 20 rpm. The refractive index was determined using an Abbe refractometer to assess the optical clarity and isotropic nature of the nanoemulsion. The cloud point was measured by gradually heating the diluted nanoemulsion and recording the temperature at which turbidity first appeared.

Self-Emulsification and Dispersibility Studies
The dispersibility and self-emulsification behavior of the formulations were evaluated using the USP Dissolution Apparatus Type II (paddle method) containing 500 mL of distilled water maintained at 37 ± 0.5°C with a paddle speed of 50 rpm. One milliliter of nanoemulsion was introduced into the dissolution medium, and the formulations were

visually assessed for clarity, dispersibility, and emulsification time. The formulations were graded according to the rate of emulsification and visual appearance.

Thermodynamic Stability Studies
The physical stability of the nanoemulsions was evaluated by heating–cooling cycles (4–40°C), centrifugation (3500 rpm for 30 min), and freeze–thaw cycles (-20°C to room temperature). Formulations showing no evidence of phase separation, creaming, precipitation, or drug crystallization were considered thermodynamically stable.

Drug Content Determination
Drug content was determined by dissolving an accurately measured quantity of nanoemulsion equivalent to the required amount of Cefixime Trihydrate in ethanol, followed by filtration through a 0.45 µm membrane filter. The absorbance was measured using a UV-visible spectrophotometer at the predetermined λmax, and the drug content was calculated from the calibration curve.

Droplet Size and Zeta Potential
The mean droplet size, polydispersity index (PDI), and zeta potential of the optimized nanoemulsion were determined using a Malvern Zetasizer after suitable dilution with distilled water. These parameters were used to evaluate droplet uniformity and colloidal stability.

FTIR and DSC Analysis
Fourier Transform Infrared (FTIR) spectroscopy and Differential Scanning Calorimetry (DSC) were performed to investigate drug–excipient compatibility and the physical state of Cefixime Trihydrate in the optimized formulation. FTIR spectra were recorded over 4000–400 cm^{-1}, while DSC thermograms were obtained by heating the samples at 10°C/min under a nitrogen atmosphere.

Surface Morphology
The surface morphology of the optimized nanoemulsion was examined using Scanning Electron Microscopy (SEM). Freeze-dried samples were sputter-coated with gold and observed under different magnifications to evaluate particle morphology and aggregation.

***In Vitro* Drug Release**
The in vitro drug release study was performed using the USP Dissolution Apparatus Type II (paddle method) in 900 mL of phosphate buffer (pH 6.8) maintained at 37 ± 0.5°C with a paddle speed of 50 rpm. Samples were withdrawn at predetermined intervals, filtered, and analyzed by UV-visible spectrophotometry at the λmax of Cefixime Trihydrate. The cumulative percentage drug release was calculated and plotted against time.

Stability Studies
The stability of the optimized nanoemulsion was evaluated according to ICH guidelines by storing the formulation at 40 ± 2°C/75 ± 5% RH for 90 days. Samples were analyzed at 30, 60, and 90 days for changes in physical appearance, phase separation, pH, drug content, and *in vitro* drug release to assess formulation stability.

RESULTS AND DISCUSSION:

Preformulation Studies
Preformulation studies were carried out to evaluate the physicochemical characteristics of Cefixime Trihydrate and to establish its suitability for the development of a nanoemulsion formulation. The studies included the evaluation of physical characteristics, melting point, and solubility profile, which are essential parameters for the selection of formulation components and optimization of the delivery system.

Physical Characteristics, Melting Point, and Solubility
Cefixime Trihydrate was observed as a white to off-white crystalline, free-flowing powder with a slightly bitter taste and no characteristic odor. The absence of discoloration, moisture, or visible impurities indicated the good quality and purity of the drug sample, making it suitable for pharmaceutical formulation.
The melting point of Cefixime Trihydrate was found to be 220–224°C, which closely agrees with the reported literature values. The narrow melting range confirmed the crystalline nature and high purity of the drug without significant degradation or contamination.

The solubility study demonstrated that Cefixime Trihydrate possesses poor aqueous solubility (0.30 ± 0.02 mg/mL), which may limit its oral bioavailability. In contrast, the drug exhibited significantly higher solubility in organic solvents and surfactants, including ethanol (15.2 ± 0.5 mg/mL), propylene glycol (12.8 ± 0.4 mg/mL), oleic acid (10.5 ± 0.3 mg/mL), and Tween 80 (9.0 ± 0.3 mg/mL). These findings justified the development of a nanoemulsion system to enhance the solubility and dissolution characteristics of Cefixime Trihydrate. The higher solubility in lipophilic vehicles also supported the selection of suitable oil and surfactant systems during formulation development.

Table 1. Preformulation Characteristics of Cefixime Trihydrate

Parameter	Observation/Result
Appearance	White to off-white crystalline powder
Odor	Odorless
Taste	Slightly bitter
Texture	Crystalline and free-flowing
Moisture Content	Negligible
Melting Point (°C)	220–224
Solubility in Water (mg/mL)	0.30 ± 0.02
Solubility in Ethanol (mg/mL)	15.2 ± 0.5
Solubility in Propylene Glycol (mg/mL)	12.8 ± 0.4
Solubility in Oleic Acid (mg/mL)	10.5 ± 0.3
Solubility in Tween 80 (mg/mL)	9.0 ± 0.3

The preformulation investigations confirmed that Cefixime Trihydrate possesses the physicochemical characteristics required for nanoemulsion formulation. Although the drug exhibited limited aqueous solubility, its significantly improved solubility in organic solvents and surfactants indicated the feasibility of developing a lipid-based nanoemulsion to enhance its dissolution and oral bioavailability. Furthermore, the observed physical characteristics and melting point confirmed the identity, purity, and thermal stability of the drug, providing a strong foundation for subsequent formulation optimization and characterization studies. This comprehensive preformulation evaluation justified the selection of the nanoemulsion approach for improving the pharmaceutical performance of Cefixime Trihydrate.

Analytical Methodology

UV-Visible Spectrophotometric Analysis

The UV-visible spectrophotometric method was developed for the quantitative estimation of Cefixime Trihydrate in ethanol. The drug exhibited a maximum absorption wavelength (λmax) at 314 nm. Standard solutions in the concentration range of 2–10 µg/mL obeyed Beer–Lambert's law and showed a linear relationship between concentration and absorbance. The calibration curve demonstrated excellent linearity with a regression equation of Y = 1.7857x + 2.8571 and a correlation coefficient (R^2) of 0.9766, confirming the suitability of the method for the estimation of drug content and in vitro drug release studies.

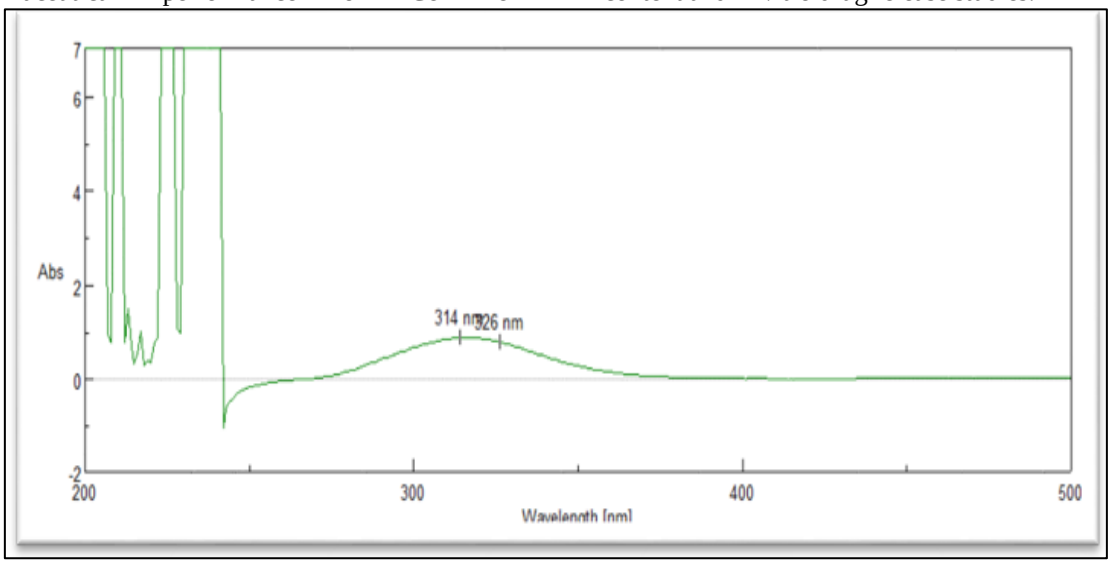

Figure 1. λmax of Cefixime Trihydrate in ethanol

Drug–Excipient Compatibility Studies

Fourier Transform Infrared (FTIR) Spectroscopy

Drug–excipient compatibility was evaluated using FTIR spectroscopy in the spectral range of 4000–400 cm^{-1}. The characteristic absorption peaks of pure Cefixime Trihydrate were compared with those of the drug–excipient physical mixtures. No significant shift, disappearance, or appearance of additional peaks was observed, indicating the absence of chemical interactions between the drug and the selected formulation excipients. The results confirmed the compatibility of Cefixime Trihydrate with Mentha oil, Tween 80, and PEG 200, demonstrating their suitability for the development of a stable nanoemulsion formulation.

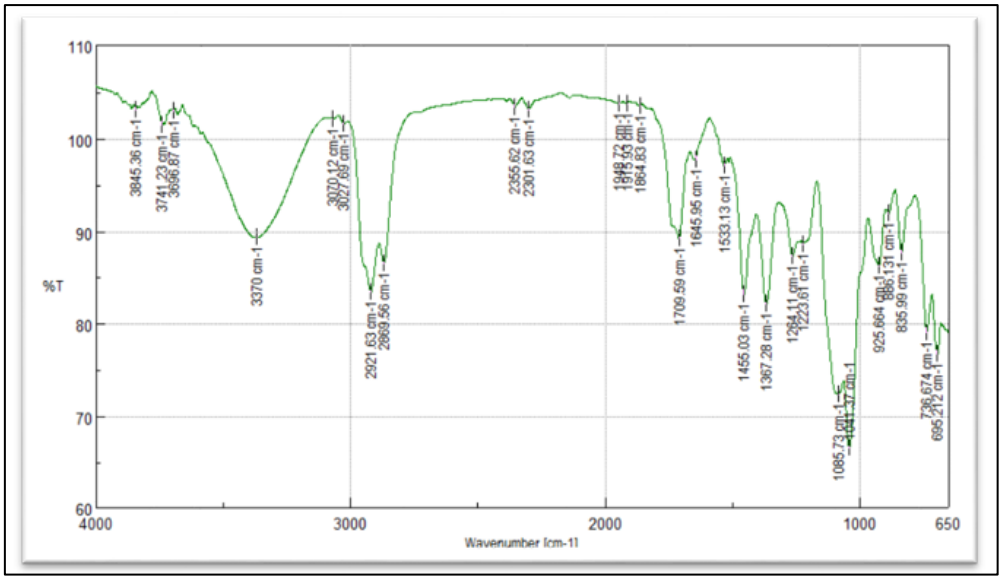

Figure 2. FT-IR Spectra of Physical mixture

Differential scanning calorimetry (DSC)

The DSC thermogram of the pure drug Cefixime Trihydrate and physical mixture of drug and other excipient are shown in following figures. The characteristic endothermic peak value of pure drug and physical mixture are shown in the figures. The characteristic peak of Cefixime Trihydrate at 250.10oC provided the endothermic melting value of the pure drug. The physical mixture of the pure drug and the polymers were used to determine the drug polymer compatibility study. The mixture showed an endothermic peak at 282.08ºC.

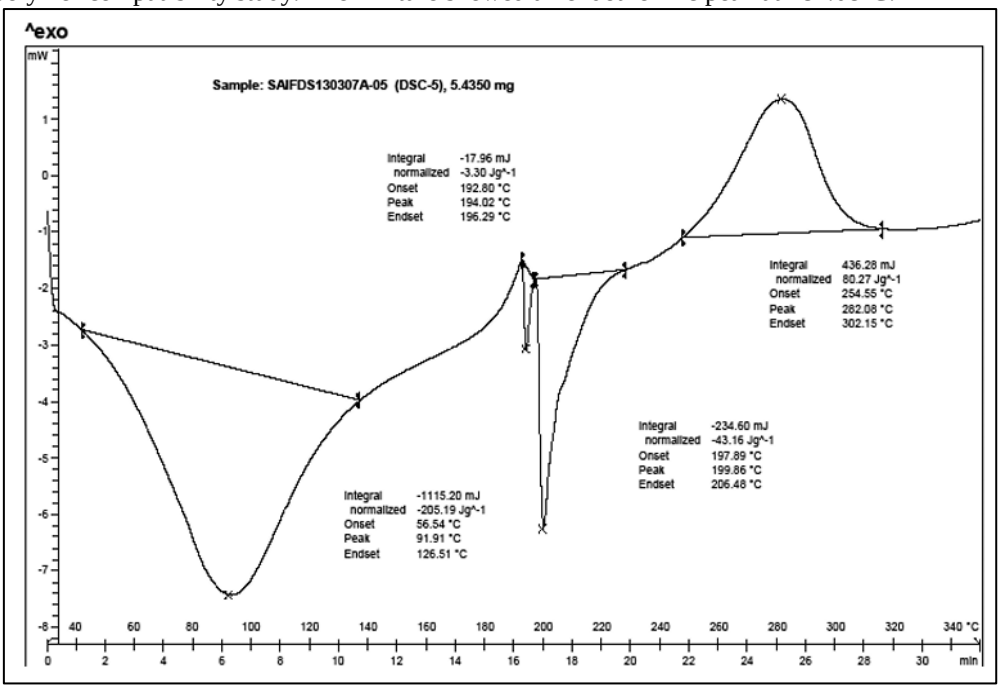

Figure 3. DSC thermogram of physical mixture

Solubility Studies

The solubility of Cefixime Trihydrate was evaluated in different oils, surfactants, and co-surfactants to identify the most suitable components for nanoemulsion development. Among the tested oils, Mentha oil exhibited the highest drug solubility (50.40 mg/mL) compared to peppermint oil, eucalyptus oil, and oleic acid. Similarly, Tween 80 showed the highest solubilizing capacity (31.50 mg/mL) among the surfactants, while PEG 200 demonstrated superior solubility (56.23 mg/mL) compared to PEG 400. Based on these findings, Mentha oil, Tween 80, and PEG 200 were selected as the oil phase, surfactant, and co-surfactant, respectively, for further formulation studies due to their excellent drug solubilization capacity.

Table 2. Solubility of Cefixime Trihydrate in Different Vehicles

Vehicle Type	Vehicle	Solubility (mg/mL)	Selection
Oil	Peppermint oil	21.99	No
	Mentha oil	50.40	Selected
	Eucalyptus oil	19.04	No
	Oleic acid	22.85	No
Surfactant	Tween 80	31.50	Selected
	Tween 20	10.20	No
	Span 80	7.57	No
	Span 20	19.51	No
Co-surfactant	PEG 400	18.90	No
	PEG 200	56.23	Selected

Pseudo-Ternary Phase Diagram Study

Pseudo-ternary phase diagrams were constructed using the aqueous titration method to determine the optimum composition range for nanoemulsion formation. The system consisted of Mentha oil, Tween 80, PEG 200, and distilled water. Among the investigated surfactant/co-surfactant (Smix) ratios, the 1:1 Smix ratio exhibited a comparatively larger nanoemulsion region, indicating better emulsification efficiency and greater formulation flexibility. In contrast, the 2:1 Smix ratio produced a relatively smaller nanoemulsion region, suggesting reduced nanoemulsion-forming capability. Therefore, the 1:1 Smix ratio was selected for subsequent formulation studies.

Optimization of Nanoemulsion Composition

Selection of Smix Ratio

Different Smix ratios (1:1, 2:1, and 1:2) were evaluated based on visual appearance and physical stability. The 1:1 Smix ratio produced a clear, transparent, and physically stable system without any evidence of phase separation, whereas the 2:1 and 1:2 ratios resulted in highly viscous formulations. Accordingly, the 1:1 Smix ratio was selected for further optimization.

Table 3. Selection of Optimum Smix Ratio

Smix Ratio	Observation	Result
1:1	Clear and stable nanoemulsion	Selected
2:1	Thick and viscous system	Rejected
1:2	Thick and viscous system	Rejected

Selection of Oil:Smix Ratio

Various Oil:Smix ratios (1:9–9:1) were investigated using the optimized 1:1 Smix ratio. Formulations with Oil:Smix ratios of 5:5, 7:3, 8:2, and 9:1 produced clear and stable nanoemulsions without phase separation, whereas the remaining formulations showed phase separation or excessive viscosity. Among these, the 5:5 Oil:Smix ratio exhibited superior clarity and physical stability and was selected for further drug loading and characterization.

Table 4. Selection of Optimum Oil:Smix Ratio

Oil:Smix Ratio	Observation	Result
1:9	Phase separation	Rejected
2:8	Phase separation	Rejected
3:7	Phase separation	Rejected
4:6	Viscous system	Rejected
5:5	Clear and stable nanoemulsion	Selected
6:4	Phase separation	Rejected
7:3	Clear and stable	Acceptable
8:2	Clear and stable	Acceptable
9:1	Clear and stable	Acceptable

The solubility and phase behavior studies demonstrated that Mentha oil, Tween 80, and PEG 200 provided the highest drug solubilization and favorable emulsification characteristics for Cefixime Trihydrate. The pseudo-ternary phase diagram confirmed that a 1:1 Smix ratio generated the largest nanoemulsion region, while optimization studies identified the 5:5 Oil:Smix ratio as the most suitable formulation composition based on clarity, homogeneity, and physical stability. These optimized formulation variables were therefore selected for the preparation and characterization of the Cefixime Trihydrate nanoemulsion.

Optimization of Drug-Loaded Nanoemulsion

Evaluation of Preliminary Drug-Loaded Nanoemulsion

Drug-loaded nanoemulsion formulations were prepared by incorporating 500 mg of Cefixime Trihydrate into the optimized oil–Smix systems. Among the four formulations investigated, A8 and A9 produced clear and homogeneous nanoemulsions without phase separation, whereas A5 and A7 exhibited comparatively higher viscosity. The preliminary study demonstrated that the optimized oil and Smix combinations were capable of incorporating Cefixime Trihydrate successfully, and the selected formulations were further evaluated for their physicochemical characteristics.

Table 5. Preliminary Evaluation of Drug-Loaded Nanoemulsion

Batch	Observation	Result
A5	Viscous	Acceptable
A7	Viscous	Acceptable
A8	Clear and stable	Selected
A9	Clear and stable	Selected

Evaluation of Cefixime Trihydrate Nanoemulsion

Physicochemical Evaluation

The prepared nanoemulsions exhibited satisfactory physicochemical characteristics. The pH ranged from 7.30 to 8.48, indicating suitability for oral administration. Density values (0.467–0.713 g/cm³) and viscosity (1.25–1.68 cP) confirmed the low-viscosity nature of the nanoemulsions. Formulations A5 and A7 exhibited excellent self-emulsification behavior with rapid emulsification times and high clarity, while A8 and A9 required comparatively longer emulsification times.

Thermodynamic stability studies revealed that formulations A5, A7, and A8 remained stable under heating–cooling, centrifugation, and freeze–thaw stress conditions, whereas A9 showed instability. High transmittance values (>99%) observed for A5 and A7 confirmed the formation of optically transparent nanoemulsions. Refractive index values between 1.506 and 1.527 further demonstrated the isotropic and homogeneous nature of the developed formulations.

Table 6. Physicochemical Evaluation of Nanoemulsion Formulations

Parameter	A5	A7	A8	A9
pH	7.83	7.30	8.37	8.48
Density (g/cm³)	0.467	0.713	0.579	0.608
Viscosity (cP)	1.49	1.25	1.68	1.61
Self-emulsification Grade	B	A	C	C
Emulsification Time (min)	0.49	0.18	1.44	1.18
Thermodynamic Stability	Pass	Pass	Pass	Fail
% Transmittance	99.17	99.87	96.00	95.12
Cloud Point (°C)	74	70	80	78
Refractive Index	1.509	1.506	1.527	—

Drug Content, Particle Size and Zeta Potential

Drug content analysis demonstrated uniform drug incorporation in all formulations, with values ranging from 85.16% to 99.62%. Formulation A7 exhibited the highest drug content (99.62 ± 0.147%), indicating efficient drug loading. Particle size analysis confirmed nanosized droplets (132.8–165.4 nm), with formulation A7 showing the smallest particle size (132.8 nm), which is advantageous for improving dissolution and oral absorption.

All formulations exhibited negative zeta potential values between −32.3 and −40.6 mV, indicating good electrostatic stability. Although formulation A8 showed the highest zeta potential magnitude, formulation A7 demonstrated the best overall balance of particle size, drug loading, and physicochemical properties.

Table 7. Drug Content and Particle Characterization

Batch	Drug Content (%)	Particle Size (nm)	Zeta Potential (mV)
A5	96.72 ± 0.09	145.2	-37.4
A7	99.62 ± 0.15	132.8	-32.3
A8	88.24 ± 0.08	165.4	-40.6
A9	85.16 ± 0.34	158.9	-38.5

SEM analysis revealed nearly spherical nanodroplets with smooth surfaces and no evidence of particle aggregation, confirming successful nanoemulsion formation.

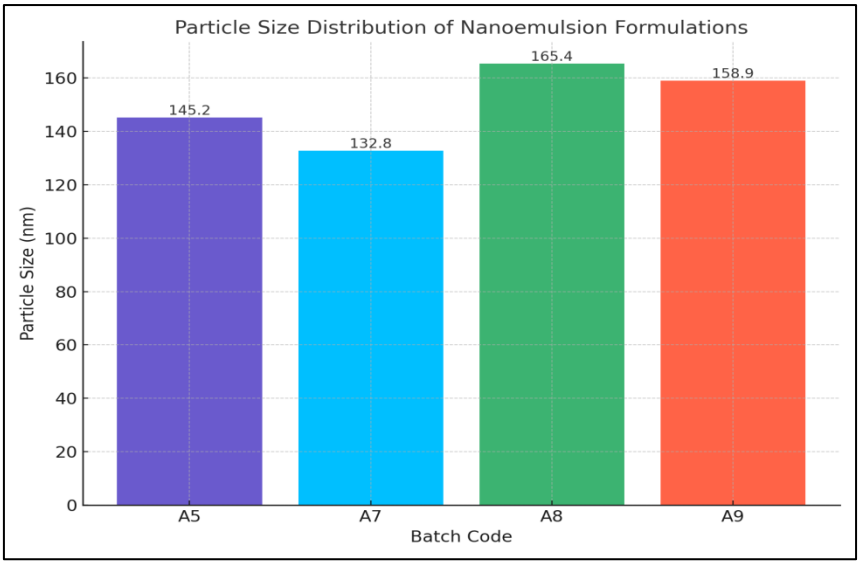

Figure 4. Particle Size Analysis of Nanoemulsion Formulations

***In Vitro* Drug Release**

The optimized nanoemulsion formulations exhibited significantly enhanced drug release compared with the pure drug. Among all formulations, A7 showed the highest cumulative drug release (98.94%) within 60 min, followed by A5 (98.64%), whereas the pure drug released only 58.65% during the same period. The rapid drug release observed for formulation A7 can be attributed to its smaller particle size, high drug loading, and efficient self-emulsification characteristics, which collectively increased the available surface area for dissolution.

Table 8. Cumulative Drug Release at 60 min

Formulation	Drug Release (%)
Pure Cefixime	58.65
A5	98.64
A7	98.94
A8	92.74
A9	93.98

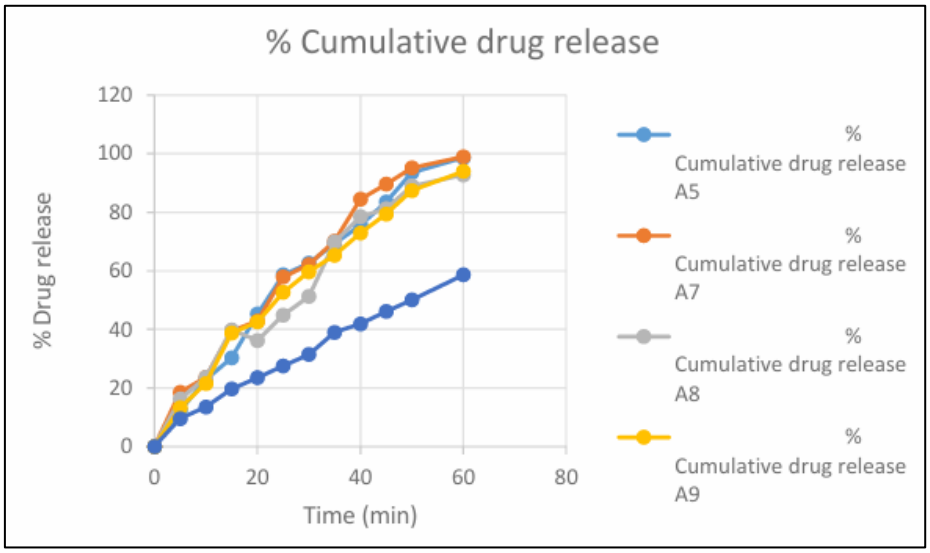

Figure 5. Graphical representation of percent drug released from the formulation

Stability Studies

Accelerated stability studies of the optimized formulation (A7) were performed according to ICH guidelines for 90 days. No visible changes such as phase separation, precipitation, or discoloration were observed throughout the study period. Drug content remained above 98%, demonstrating excellent physical and chemical stability of the optimized nanoemulsion.

The optimization studies demonstrated that formulation A7 possessed the most desirable characteristics among all investigated formulations. It exhibited excellent self-emulsification, high drug content, nanosized droplets, satisfactory zeta potential, rapid *in vitro* drug release, and excellent

storage stability. These findings indicate that the developed Cefixime Trihydrate nanoemulsion has strong potential as an oral drug delivery system for improving the solubility, dissolution rate, and bioavailability of Cefixime Trihydrate.

CONCLUSION:

The present investigation successfully developed and optimized a Cefixime Trihydrate nanoemulsion using Mentha oil, Tween 80, and PEG 200 as the formulation components. Preformulation and compatibility studies confirmed the purity of the drug and its compatibility with the selected excipients, providing a suitable foundation for nanoemulsion development. The optimized formulation (Batch A7) exhibited desirable physicochemical characteristics, including appropriate pH, low viscosity, excellent self-emulsification behavior, high drug content (99.62%), nanosized droplets (132.8 nm), and adequate zeta potential (−32.3 mV), indicating good formulation stability. The nanoemulsion significantly enhanced the dissolution performance of Cefixime Trihydrate, achieving approximately 98.94% drug release within 60 minutes, compared with 58.65% from the pure drug. The improved dissolution can be attributed to the reduced droplet size, increased interfacial surface area, and efficient dispersion of the drug within the nanoemulsion system. Furthermore, accelerated stability studies demonstrated that the optimized formulation remained physically and chemically stable for 90 days under ICH storage conditions without significant changes in drug content or appearance. Overall, the developed nanoemulsion represents a promising lipid-based oral drug delivery system capable of overcoming the poor aqueous solubility of Cefixime Trihydrate. The formulation has the potential to improve oral bioavailability, therapeutic efficacy, and patient compliance. Future studies should focus on *in vivo* pharmacokinetic, pharmacodynamic, and bioavailability evaluations, followed by clinical investigations to confirm the therapeutic advantages of the developed nanoemulsion.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

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