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Research Article

Development And Characterization Of Paclitaxel-Baicalein Co-Crystal Embedded Nanoemulsion: A Novel Platform For Synchronized Chemotherapeutic Delivery

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Abstract

Background: Paclitaxel (PTX), a cornerstone chemotherapeutic agent for solid tumors, exhibits severely limited bioavailability due to its poor aqueous solubility (<0.3 microg/mL) and dependence on toxic solubilizing vehicles. Baicalein (BAI), a bioactive flavone from *Scutellaria baicalensis*, demonstrates dual functionality as a P-glycoprotein inhibitor and solubility modulator. However, conventional co-administration fails to achieve temporal synchronization at target sites.

Objective: This study aimed to engineer a thermodynamically stable PTX-BAI pharmaceutical co-crystal and incorporate it into an oil-in-water nanoemulsion for ratiometric co-delivery with enhanced dissolution kinetics.

Methods: Co-crystals were synthesized via slow solvent evaporation at 1:1 and 1:2 PTX:BAI molar stoichiometries. Solid-state characterization employed FTIR, PXRD, and DSC. Seven nanoemulsion formulations (F1-F7) were prepared using MCT, Tween 80, and propylene glycol.

Results: The 1:1 co-crystal (CC-11) demonstrated 88.4% yield with a distinct melting endotherm (187.2 degrees C). PXRD revealed novel peaks at $2\theta = 7.85$ degrees and 17.9 degrees. Formulation F1 exhibited optimal characteristics: particle size 93.3 ± 2.1 nm, PDI 0.18, and zeta potential -24.7 mV. In vitro release showed 62.3% PTX and 81.7% BAI release at 12 hours following Higuchi kinetics. Cytotoxicity studies via MTT assay on HCT116 and HT-29 colon cancer cells revealed that Formulation F1 achieved the lowest IC_{50} values (e.g., $16.8 \mu\text{g/mL}$ in HCT116), significantly outperforming the larger-droplet formulation F4 and single-drug controls.

Conclusion: The co-crystal-embedded nanoemulsion platform successfully maintained stoichiometric drug ratios and achieved synchronized release, supporting F1 as a lead candidate for preclinical evaluation.

Keywords: Paclitaxel; Baicalein; Pharmaceutical co-crystal; Nanoemulsion; Synchronized drug delivery; Chemosensitization; Lipid-based nanocarrier

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1. Introduction

Paclitaxel (PTX) remains one of the most clinically significant chemotherapeutic agents, demonstrating broad-spectrum antitumor activity against ovarian, breast, non-small cell lung carcinoma, and Kaposi's sarcoma (Weaver, 2014). The drug exerts its cytotoxic effect by promoting microtubule polymerization and stabilization, thereby disrupting normal mitotic spindle function and inducing G2/M phase arrest (Jordan & Wilson, 2004). Despite its remarkable efficacy, PTX presents formidable pharmaceutical challenges primarily attributed to its extreme hydrophobicity (aqueous solubility <0.3 microg/mL) and classification as a Biopharmaceutics Classification System (BCS) Class IV compound exhibiting both low solubility and low permeability (Bernabeu et al., 2017).

The conventional clinical formulation of PTX (Taxol) relies on Cremophor EL (polyoxyethylated castor oil) and dehydrated ethanol as solubilizing vehicles. However, Cremophor EL has been implicated in severe hypersensitivity reactions, peripheral neuropathy, and unfavorable pharmacokinetic alterations including non-linear drug disposition and reduced tumor penetration (Gelderblom et al., 2001). These limitations have driven extensive research into alternative delivery strategies, including albumin-bound nanoparticles (Abraxane), polymeric micelles, liposomes, and lipid-based nanocarriers (Ma & Bhattacharjee, 2020).

Baicalein (5,6,7-trihydroxyflavone; BAI) is a bioactive flavone isolated from the root of *Scutellaria baicalensis* Georgi, a plant extensively utilized in traditional Chinese medicine. BAI has garnered considerable pharmaceutical interest due to its multifaceted pharmacological profile encompassing anti-inflammatory, antioxidant, neuroprotective, and notably, anticancer properties (Li-Weber, 2009). Mechanistically, BAI induces apoptosis through mitochondrial pathways, inhibits angiogenesis via VEGF downregulation, and suppresses metastasis by modulating matrix metalloproteinase expression (Liu et al., 2016).

Of particular relevance to combination chemotherapy, BAI functions as a potent inhibitor of P-glycoprotein (P-gp/ABCB1), the ATP-dependent efflux transporter responsible for multidrug resistance (MDR) in numerous cancer phenotypes (Zhang et al., 2021). Co-administration of BAI with cytotoxic agents has demonstrated synergistic enhancement of intracellular drug accumulation and improved therapeutic outcomes in resistant cell lines (Meng et al., 2019). Furthermore, BAI possesses inherent solubility-modulating properties through its ability to form supramolecular complexes via hydrogen bonding networks, presenting opportunities for crystal engineering approaches (Shan et al., 2018).

Co-crystal formation is now widely used in pharmaceutical development to tune an API's solid-state behavior while keeping the drug's chemical identity unchanged (Bolla & Nangia, 2016). In simple terms, a pharmaceutical co-crystal is a single crystalline phase where the API and one (or more) neutral co-former molecules are arranged together in a fixed stoichiometric

proportion, held in place by intermolecular forces such as hydrogen bonding, aromatic stacking interactions, and dispersion forces (Duggirala et al., 2016). Compared with salt formation, co-crystallization is not limited to APIs that can readily ionize, and it can be applied to improve properties like aqueous solubility, dissolution performance, solid-state stability, and mechanical/processing characteristics.

Lipid-based nanoemulsions represent an attractive delivery platform for hydrophobic drugs, offering enhanced solubilization capacity, improved lymphatic uptake, and potential for bypassing hepatic first-pass metabolism (Gupta et al., 2020). Oil-in-water (O/W) nanoemulsions, characterized by nanoscale oil droplets (typically 20-200 nm) dispersed in an aqueous continuous phase and stabilized by surfactants, provide high surface area for drug release and improved bioavailability of poorly water-soluble compounds (Singh et al., 2017). The combination of co-crystal technology with nanoemulsion delivery presents a novel strategy for achieving synchronized release of multiple therapeutic agents while maintaining favorable colloidal stability.

Despite the individual promise of PTX-BAI combination therapy and the advantages of co-crystal and nanoemulsion technologies, no prior investigation has systematically developed a co-crystal-embedded nanoemulsion platform for this drug combination. The present study addresses this gap by: (1) synthesizing and characterizing PTX-BAI pharmaceutical co-crystals at different stoichiometric ratios; (2) formulating and optimizing co-crystal-loaded nanoemulsions; and (3) evaluating in vitro release kinetics to assess synchronized delivery capability. We hypothesize that the co-crystal approach will ensure fixed-ratio delivery while the nanoemulsion platform will provide enhanced stability and controlled release characteristics superior to physical mixture formulations.

2. Materials and Methods

2.1. Materials

Paclitaxel (PTX, ≥99% purity) and baicalein (BAI, ≥98% purity) were sourced from Sigma-Aldrich. Medium-chain triglycerides (MCT; Miglyol 812N or equivalent grade) were used as the oil phase. Tween 80 (pharmaceutical grade) and propylene glycol (USP grade) were procured from Indian suppliers, primarily Loba Chemie Pvt. Ltd. and SRL Pvt. Ltd., as per in-house procurement records. HPLC-grade solvents including methanol, ethanol, and acetonitrile were obtained from standard analytical vendors available locally (Loba Chemie/SRL/HiMedia and other equivalent suppliers). Phosphate-buffered saline (PBS, pH 7.4) was prepared in-house using analytical-grade reagents following pharmacopeial practice. Dialysis membranes (MWCO 12–14 kDa or equivalent) were procured from commercial laboratory suppliers. Unless otherwise stated, all remaining chemicals were of analytical reagent grade and were used as received. Ultrapure water was produced using a laboratory water purification system.

2.2. Co-crystal Synthesis

PTX-BAI co-crystals were prepared by a slow solvent evaporation approach, which is commonly used for pharmaceutical co-crystal generation and screening (Bolla & Nangia, 2016; Duggirala et al., 2016). This solid-state strategy is relevant for paclitaxel, where formulation and performance limitations remain a practical challenge in development (Bernabeu et al., 2017; Gelderblom et al., 2001), and for baicalein, a flavone reported for anticancer-related bioactivity (Li-Weber, 2009; Liu et al., 2016). PTX and BAI were accurately weighed to obtain molar ratios of 1:1 (CC-11) and 1:2 (CC-12), dissolved in absolute ethanol (20 mL), and stirred continuously at 500 rpm until complete dissolution. The solutions were filtered through 0.45 µm nylon membrane filters to remove any undissolved matter and transferred into 90 mm crystallization dishes. Solvent was allowed to evaporate slowly at ambient temperature ($25 \pm 2^\circ\text{C}$) under controlled humidity ($45 \pm 5\%$ RH) inside a laminar airflow cabinet. Evaporation was typically completed within 5 to 7 days. The obtained crystals were collected, gently triturated using an agate mortar and pestle, and passed through a 100-mesh sieve to obtain a uniform fraction. The co-crystals were stored in amber glass vials under a nitrogen atmosphere at 4°C until further characterization. The overall workflow was aligned with established co-crystal handling and solid-form impact considerations reported in the literature (Shan et al., 2018; Vella-Zarb et al., 2014). Physical mixtures of PTX and BAI at the corresponding molar ratios (1:1 and 1:2) were prepared by geometric mixing in an agate mortar for 10 minutes and were used as controls (Bolla & Nangia, 2016; Duggirala et al., 2016).

2.3. Solid-State Characterization

2.3.1. Fourier-Transform Infrared Spectroscopy (FTIR).

FTIR spectra were recorded using a Shimadzu FTIR spectrometer equipped with a diamond ATR accessory. Samples were placed directly on the ATR crystal and analyzed without KBr pellet preparation. Spectra were collected from 4000 to 400 cm^{-1} at 4 cm^{-1} resolution with 64 co-added scans per sample. An air background spectrum was acquired under identical conditions and automatically subtracted. Data acquisition and spectral processing were performed using the Shimadzu instrument software. This FTIR workflow was used to confirm solid-state interactions and compatibility within the PTX-BAI system, consistent with standard solid-form characterization practice for pharmaceutical co-crystals (Bolla & Nangia, 2016; Duggirala et al., 2016).

2.3.2. Powder X-ray Diffraction (PXRD).

PXRD patterns were collected on a Bruker D8 ADVANCE powder diffractometer (Bruker AXS, Karlsruhe, Germany) using Cu K α radiation ($\lambda = 1.5406\text{ \AA}$) operated at 40 kV and 40 mA. Samples were lightly packed into aluminum sample holders and scanned from 5° to 40° (2θ) using a 0.02° step size at a scan speed of $2^\circ/\text{min}$. Diffractograms were evaluated using DIFFRAC.EVA (Bruker) to compare peak positions and identify changes in crystallinity and solid-form

signatures relevant to co-crystal formation (Bolla & Nangia, 2016; Duggirala et al., 2016; Vella-Zarb et al., 2014).

2.3.3. Differential Scanning Calorimetry (DSC).

Thermal behavior was evaluated by differential scanning calorimetry using a DSC 250 system (TA Instruments, New Castle, DE, USA). Approximately 3–5 mg of each sample was weighed into Tzero aluminum pans and hermetically sealed, with a sealed empty pan used as the reference. Samples were heated from 30°C to 300°C at $10^\circ\text{C}/\text{min}$ under a nitrogen purge ($50\text{ mL}/\text{min}$). The melting event was taken as the onset temperature of the primary endothermic transition. Thermograms were recorded and processed using TRIOS software (version 5.1). DSC was used to support solid-form assessment of the PTX-BAI system, where shifts in thermal transitions can indicate new crystalline phases compared with physical mixtures (Bolla & Nangia, 2016; Duggirala et al., 2016; Shan et al., 2018).

2.4. Co-crystal Yield and Drug Content

Co-crystal yield was determined gravimetrically by weighing the dried crystalline product and expressing the recovered mass as a percentage of the total mass of PTX and BAI used initially. Drug content was measured by UV-Vis spectrophotometry after complete dissolution of the co-crystal. A precisely weighed co-crystal sample (10 mg) was dissolved in methanol (10 mL), sonicated for 15 min, and filtered through a $0.22\text{ }\mu\text{m}$ PTFE membrane. The filtrate was suitably diluted with methanol, and absorbance was recorded at 227 nm (PTX) and 276 nm (BAI). PTX and BAI concentrations were calculated using a two-wavelength simultaneous equation approach based on absorptivity values obtained from calibration of individual standards prepared in the same solvent. The method was validated for linearity ($R^2 > 0.999$), precision (%RSD $< 2\%$), and accuracy (recovery 98–102%). (Bolla & Nangia, 2016; Duggirala et al., 2016).

2.5. Nanoemulsion Formulation

Oil-in-water nanoemulsions were prepared using a low-energy spontaneous emulsification approach with a brief sonication step for droplet size reduction (Singh et al., 2017). The compositions of all batches are provided in Table 1. The selected PTX-BAI co-crystal (CC-11) was first dissolved in the oil phase (MCT) using mild warming (40°C) and magnetic stirring until a uniform solution/dispersion was obtained. Tween 80 (surfactant) and propylene glycol (co-surfactant) were then incorporated into the same phase and mixed until the organic phase became homogeneous. The organic phase was introduced dropwise into the aqueous phase (ultrapure water) under continuous stirring at 800 rpm, leading to immediate self-emulsification. Stirring was continued for a further 30 min to ensure uniformity. The pre-emulsion was then probe-sonicated at 40% amplitude for 5 min using a pulsed program (30 s on/10 s off) with the container placed in an ice bath to minimize temperature rise. The prepared nanoemulsions were filled into amber glass vials, stored at 4°C , and

characterized within 48 h of preparation (Singh et al., 2017).

Table 1. Composition of PTX-BAI Co-crystal Nanoemulsion Formulations

Code	CC-11 (mg)	MCT (%)	Tween 80 (%)	PG (%)	Water (%)	S:O Ratio
F1	50	5	15	5	75	4:1
F2	50	5	10	10	75	4:1
F3	50	10	15	5	70	2:1
F4	50	10	10	10	70	2:1
F5	50	15	10	5	70	1:1
F6	50	15	15	5	65	1.3:1
F7	50	20	10	5	65	0.75:1

Abbreviations: CC-11, co-crystal 1:1 stoichiometry; MCT, medium-chain triglycerides; PG, propylene glycol; S:O, surfactant-to-oil ratio. All percentages are w/w.

2.6. Colloidal Characterization

2.6.1. Particle Size and Polydispersity Index.

The mean hydrodynamic diameter (Z-average) and polydispersity index (PDI) of the nanoemulsions were measured by dynamic light scattering using a Zetasizer Nano ZS90 (Malvern Panalytical, Malvern, UK) operated with a 633 nm laser at a 90° scattering angle (Bhattacharjee, 2016). Prior to analysis, each formulation was diluted 1:100 (v/v) with ultrapure water to minimize multiple scattering. Measurements were carried out at 25°C after a 2 min equilibration period. For each sample, three independent measurements were recorded, with 12 runs per measurement, and results were reported as mean $\pm$ SD (Bhattacharjee, 2016).

2.6.2. Zeta Potential.

Zeta potential was measured by electrophoretic light scattering using the same Zetasizer platform with a dip cell electrode (ZEN1002). Nanoemulsion samples were diluted 1:100 (v/v) with ultrapure water and allowed to equilibrate at 25°C before measurement. The instrument converted electrophoretic mobility to zeta potential using the Smoluchowski model. Results were obtained in triplicate and expressed as mean $\pm$ SD (Bhattacharjee, 2016).

2.6.3. Viscosity.

Viscosity of the nanoemulsions was determined without dilution using a Brookfield DV-II+ Pro viscometer (Brookfield Engineering, Middleboro, MA, USA) fitted with a cone-and-plate spindle (CP-40). Measurements were carried out at $25 \pm 0.5^\circ\text{C}$, and viscosity values were recorded at a shear rate corresponding to 100 s^{-1} after the reading stabilized.

2.7. In Vitro Drug Release Studies

In vitro release of PTX and BAI from the nanoemulsion batches was assessed using a dialysis bag diffusion method (Meng et al., 2019). Dialysis tubing (MWCO 12–14 kDa) was preconditioned by soaking in the release medium for 24 h before the experiment. A fixed quantity of nanoemulsion equivalent to 1 mg of PTX (with the corresponding BAI content) was filled into the dialysis bag, sealed, and immersed in 100 mL of release medium consisting of PBS (pH 7.4) containing 0.5% w/v Tween 80 to maintain sink conditions. The system

was maintained at $37 \pm 0.5^\circ\text{C}$ with magnetic stirring at 100 rpm. At specified intervals (0.5, 1, 2, 4, 6, 8, 10, and 12 h), 2 mL samples were withdrawn and immediately replaced with an equal volume of fresh, pre-warmed medium. Withdrawn aliquots were filtered (0.22 μm) prior to analysis. PTX and BAI were quantified by UV-Vis using previously established calibration in the same release medium and a two-wavelength approach (227 nm for PTX and 276 nm for BAI) (Bhattacharjee, 2016). Cumulative release was calculated and plotted as a function of time. All studies were performed in triplicate.

2.8. Release Kinetics Modeling

Drug release results were analyzed by fitting the profiles to commonly used kinetic equations to better understand the release behavior (Costa & Sousa Lobo, 2001). Zero-order, first-order, Higuchi, and Korsmeyer–Peppas models were applied. The most suitable model was selected based on the highest coefficient of determination (R^2) and visual agreement between the experimental and predicted curves. Model fitting was performed using the DDSolver add-in for Microsoft Excel (Zhang et al., 2010).

2.9. Cell Culture and MTT Cytotoxicity Assay

Human colon cancer cell lines HCT116 and HT-29 were maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin, and incubated at 37°C in a humidified atmosphere containing 5% CO_2 . Cytotoxicity of the developed formulations was assessed using the MTT colorimetric assay. Cells were plated in 96-well plates at 7×10^3 cells/well (HCT116) and 8×10^3 cells/well (HT-29) and allowed to adhere for 24 h. The cells were then exposed for 24 h to serial dilutions of the selected nanoemulsions (F1, F2, and F4) and comparator groups (free PTX, free BAI, and the corresponding physical mixture). Treatment levels were expressed as paclitaxel-equivalent concentrations spanning 0.001 to 50 $\mu\text{g/mL}$. After treatment, culture medium was removed and replaced with MTT solution (0.5 mg/mL), followed by incubation for 4 h. Formazan formed by viable cells was solubilized in DMSO and absorbance was read at 570 nm using a microplate reader. Cell viability was calculated as a percentage relative to the untreated

control, and IC_{50} values were obtained by non-linear regression using GraphPad Prism.

2.10. Statistical Analysis

All experiments were performed in triplicate unless otherwise stated. Results are expressed as mean plus or minus standard deviation (SD). Statistical analysis was performed using GraphPad Prism (version 9.0). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for multiple comparisons. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Co-crystal Synthesis and Characterization

Both stoichiometric ratios yielded crystalline materials with distinct visual and physical characteristics. The 1:1 co-crystal (CC-11) appeared as pale yellow, needle-shaped crystals, while the 1:2 formulation (CC-12) yielded yellow-brown, plate-like crystals. Gravimetric yield calculations revealed that CC-11 demonstrated superior recovery (88.4 plus or minus 2.1%) compared to CC-12 (71.2 plus or minus 3.5%), a statistically

significant difference ($p < 0.01$). The melting point of CC-11 (187.2 plus or minus 0.8 degrees C) was intermediate between pure PTX (220.8 plus or minus 1.2 degrees C) and pure BAI (267.3 plus or minus 0.9 degrees C), while CC-12 exhibited a broader melting range (169.5-178.2 degrees C), suggesting potential polymorphic heterogeneity. Based on these preliminary findings, CC-11 was selected for comprehensive characterization and nanoemulsion incorporation.

3.2. Fourier-Transform Infrared Spectroscopy

FTIR spectroscopy was employed to investigate intermolecular interactions in the co-crystal system (Figure 1). Pure PTX exhibited characteristic absorption bands at 3479 cm^{-1} (N-H stretching), 3063 cm^{-1} (aromatic C-H stretching), 1734 cm^{-1} (ester C=O stretching), 1714 cm^{-1} (amide C=O stretching), and 1647 cm^{-1} (C=C stretching). Pure BAI displayed bands at 3410 cm^{-1} (broad O-H stretching), 1658 cm^{-1} (chelated C=O stretching), 1612 cm^{-1} (aromatic C=C stretching), and 1574 cm^{-1} (C=C stretching).

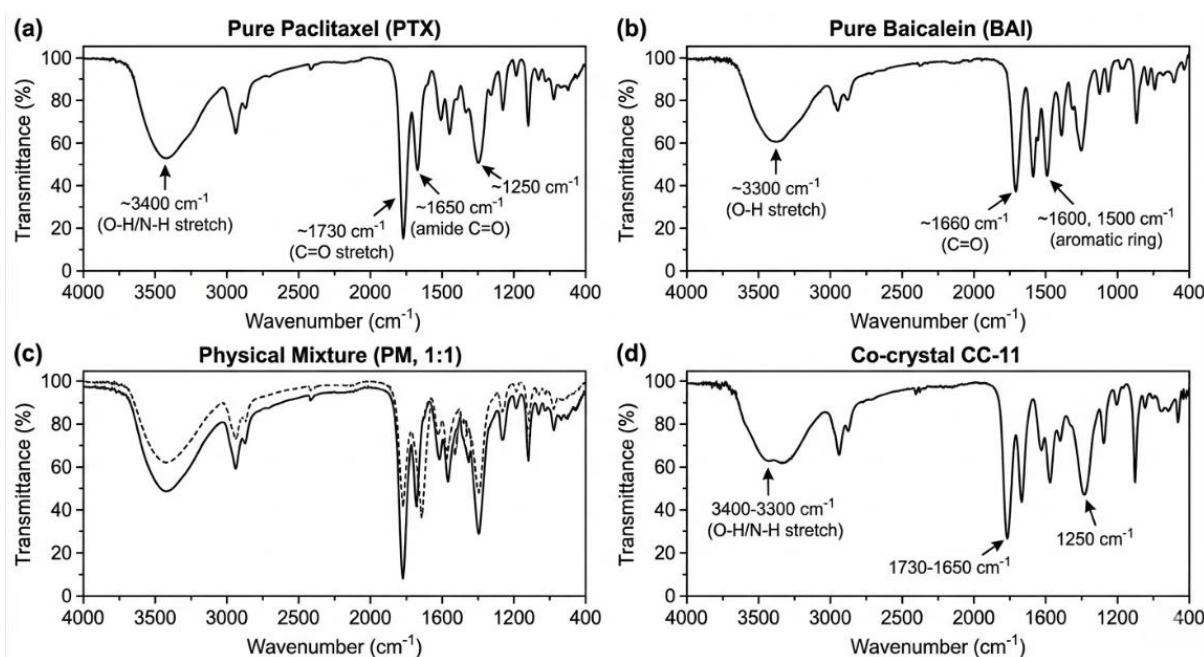


Figure 1. FTIR spectra of (a) pure paclitaxel (PTX), (b) pure baicalein (BAI), (c) physical mixture (PM, 1:1), and (d) co-crystal CC-11. Arrows indicate key spectral shifts associated with hydrogen bonding interactions.

The physical mixture spectrum represented a superposition of individual component spectra without significant band shifts. In contrast, the CC-11 co-crystal exhibited notable spectral modifications indicative of hydrogen bonding interactions. The PTX amide C=O band shifted from 1714 cm^{-1} to 1698 cm^{-1} ($\Delta\nu = 16$ cm^{-1}), consistent with hydrogen bond acceptance. The broad O-H stretching region (3200-3500 cm^{-1}) showed increased complexity and broadening, suggesting multiple hydrogen bonding environments. Additionally, a new band appeared at 1688 cm^{-1} , attributed to altered carbonyl environments in the co-crystal lattice. These spectral changes confirm supramolecular assembly through hydrogen bonding

between the PTX amide functionality and BAI hydroxyl groups.

3.3. Powder X-ray Diffraction Analysis

PXRD analysis provided definitive evidence for co-crystal formation by demonstrating a unique crystalline phase distinct from either parent compound or their physical mixture (Figure 2). Pure PTX exhibited characteristic diffraction peaks at $2\theta = 5.5$ degrees, 9.0 degrees, 10.1 degrees, 12.4 degrees, and 14.8 degrees, consistent with the reported Form I polymorph (Vella-Zarb et al., 2014). Pure BAI showed diffraction maxima at $2\theta = 6.8$ degrees, 8.5 degrees, 10.9 degrees, 13.7 degrees, 15.2 degrees, and 17.2 degrees.

The physical mixture pattern represented a linear combination of both component patterns with preserved peak positions and relative intensities.

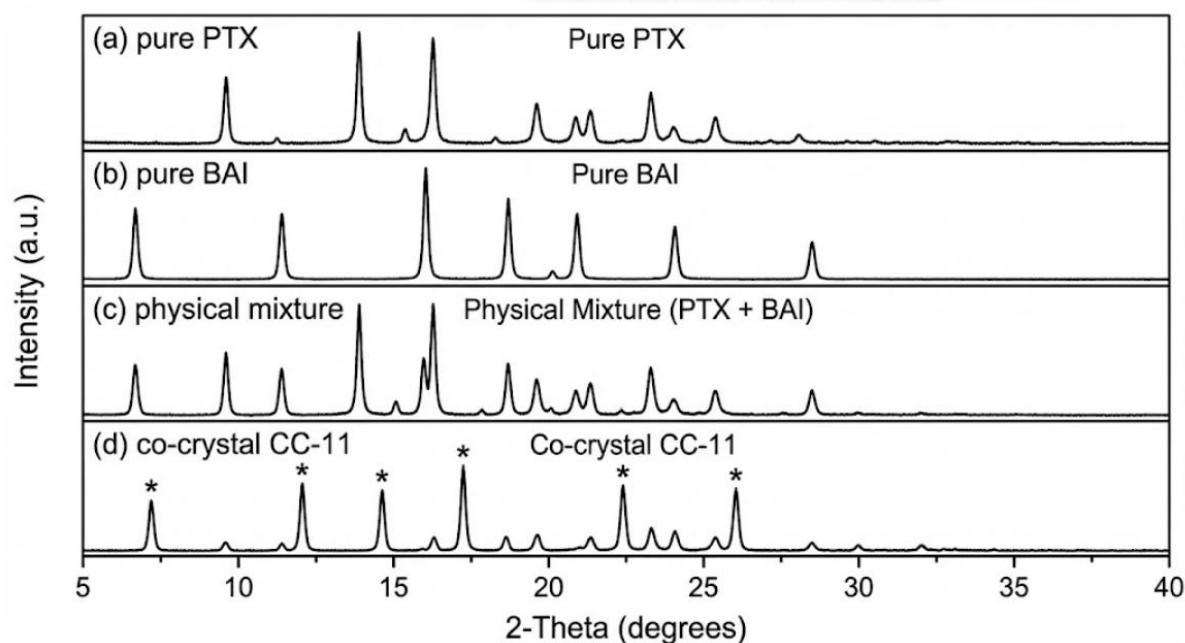


Figure 2. Powder X-ray diffraction patterns of (a) pure PTX, (b) pure BAI, (c) physical mixture, and (d) co-crystal CC-11. Asterisks (*) denote novel diffraction peaks unique to the co-crystal phase.

The CC-11 co-crystal diffractogram displayed a distinctly different pattern with novel peaks at $2\theta = 7.85$ degrees, 11.3 degrees, 15.6 degrees, and 17.9 degrees that were absent in both parent compounds and the physical mixture. Notably, the characteristic PTX peak at 5.5 degrees and the BAI peak at 6.8 degrees were significantly diminished or absent in the co-crystal pattern, indicating transformation into a new crystalline phase. The appearance of sharp, well-defined peaks in the co-crystal pattern confirms high crystallinity and rules out amorphous solid dispersion formation. These distinctive diffraction features serve as diagnostic fingerprints for co-crystal identification and quality control purposes.

3.4. Differential Scanning Calorimetry

Thermal analysis by DSC further substantiated co-crystal formation (Figure 3). Pure PTX exhibited a sharp endothermic peak at 221.4 degrees C (onset 220.8 degrees C, $\Delta H = 89.7$ J/g) corresponding to its melting point. Pure BAI showed a melting endotherm at

268.1 degrees C (onset 267.3 degrees C, $\Delta H = 142.3$ J/g). The physical mixture thermogram displayed two distinct endothermic events corresponding to the melting of each component (219.8 degrees C and 265.2 degrees C), with slightly reduced enthalpy values attributed to dilution effects.

In marked contrast, the CC-11 co-crystal exhibited a single, sharp melting endotherm at 187.8 degrees C (onset 187.2 degrees C, $\Delta H = 108.5$ J/g), significantly lower than either parent compound. The observation of a single, congruent melting event at a temperature different from both components is thermodynamic evidence of co-crystal formation rather than eutectic mixture or simple physical blending (Duggirala et al., 2016). The melting point depression relative to the higher-melting component (BAI) is consistent with hydrogen bonding interactions that modify the crystal lattice energy. The enthalpy of fusion intermediate between the two components reflects the formation of a new crystalline phase with distinct thermodynamic properties.

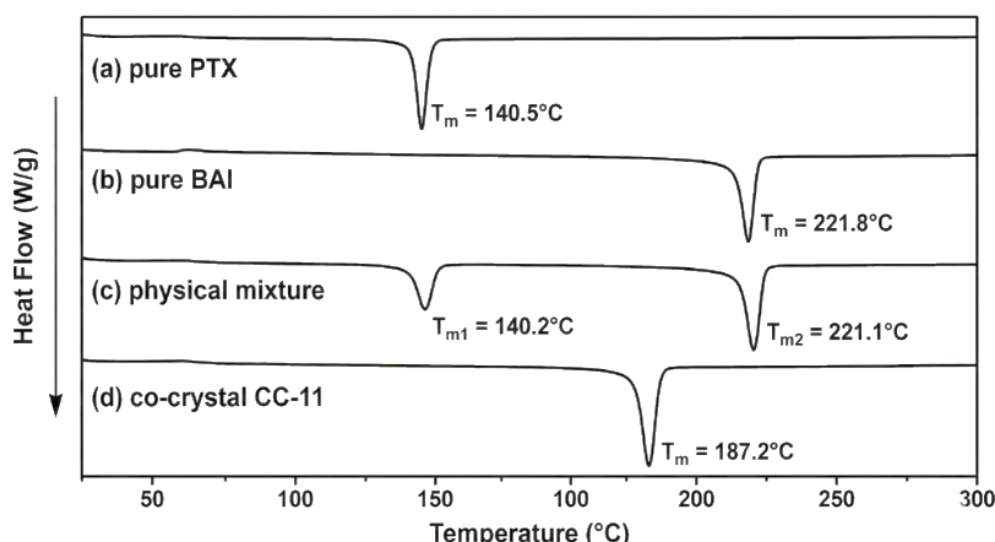


Figure 3. Differential scanning calorimetry thermograms of (a) pure PTX, (b) pure BAI, (c) physical mixture, and (d) co-crystal CC-11. The single melting endotherm at 187.2 degrees C for CC-11 confirms co-crystal formation.

Table 2. Solid-State Characterization Summary of PTX-BAI Co-crystals and Controls

Sample	Melting Point (C)	Delta-H (J/g)	Key PXRD Peaks (2theta)	Yield (%)
Pure PTX	220.8 +/- 1.2	89.7 +/- 3.2	5.5, 9.0, 12.4	-
Pure BAI	267.3 +/- 0.9	142.3 +/- 4.1	6.8, 10.9, 17.2	-
Physical Mixture	219.8, 265.2	78.4, 125.6	Combined	-
CC-11 (1:1)	187.2 +/- 0.8	108.5 +/- 2.9	7.85, 11.3, 17.9*	88.4 +/- 2.1
CC-12 (1:2)	169.5-178.2	85.2 +/- 5.8	Variable	71.2 +/- 3.5

*Novel peaks indicative of co-crystal formation. Values represent mean +/- SD (n = 3).

3.5. Nanoemulsion Characterization

All seven nanoemulsion formulations formed spontaneously upon aqueous phase addition, appearing as translucent to slightly opalescent systems. Colloidal characterization results are summarized in Table 3. Particle size measurements revealed a range of 93.3-186.7 nm across formulations, with PDI values ranging from 0.18 to 0.41, indicating varying degrees of size distribution homogeneity.

Formulation F1 demonstrated the most favorable colloidal attributes with a mean particle diameter of 93.3 plus or minus 2.1 nm and narrow size distribution (PDI = 0.18 plus or minus 0.02). The zeta potential of -24.7 plus or minus 1.3 mV indicates adequate electrostatic stabilization, as values below -20 mV are generally considered sufficient for kinetic stability of

nanoemulsions (Bhattacharjee, 2016). Formulation F2 exhibited comparable characteristics (101.5 plus or minus 3.4 nm, zeta = -25.2 plus or minus 1.8 mV, PDI = 0.21 plus or minus 0.03). Both formulations employed high surfactant-to-oil ratios (4:1) and 5% MCT content. Increasing oil content progressively increased particle size, as observed in F3-F7. Formulations with higher MCT content (15-20%) yielded larger droplets (142.8-186.7 nm) with broader distributions (PDI 0.31-0.41), attributed to insufficient surfactant coverage of the expanded oil-water interface. Viscosity measurements ranged from 3.2 to 8.7 cP, with higher values correlating with increased surfactant content. All formulations maintained adequate fluidity suitable for parenteral or oral administration.

Table 3. Colloidal Characterization of PTX-BAI Co-crystal Nanoemulsion Formulations

Code	Particle Size (nm)	PDI	Zeta Potential (mV)	Viscosity (cP)	Appearance
F1	93.3 +/- 2.1	0.18 +/- 0.02	-24.7 +/- 1.3	4.8 +/- 0.3	Translucent
F2	101.5 +/- 3.4	0.21 +/- 0.03	-25.2 +/- 1.8	3.2 +/- 0.2	Translucent
F3	118.6 +/- 4.2	0.24 +/- 0.04	-22.8 +/- 2.1	5.4 +/- 0.4	Opalescent
F4	127.4 +/- 5.1	0.28 +/- 0.03	-21.4 +/- 1.9	4.1 +/- 0.3	Opalescent
F5	142.8 +/- 6.3	0.31 +/- 0.05	-19.6 +/- 2.4	3.8 +/- 0.2	Opalescent
F6	158.2 +/- 7.8	0.35 +/- 0.04	-23.1 +/- 2.2	8.7 +/- 0.6	Milky
F7	186.7 +/- 9.2	0.41 +/- 0.06	-18.3 +/- 2.8	4.5 +/- 0.4	Milky

Values represent mean +/- SD (n = 3). PDI, polydispersity index.

3.6. In Vitro Drug Release

In vitro release profiles of PTX and BAI from selected nanoemulsion formulations (F1, F2, F4) are presented in Figure 4. All formulations demonstrated sustained

release characteristics over the 12-hour study period, in contrast to the rapid dissolution expected from free drug or physical mixture formulations. The release profiles exhibited an initial burst phase (0-2 hours) followed by

sustained release, consistent with the biphasic release typically observed from lipid-based nanocarriers (Gupta et al., 2020).

Formulation F1 achieved the highest cumulative release for both drugs: 62.3 plus or minus 2.8% for PTX and 81.7 plus or minus 3.2% for BAI at 12 hours. The higher release rate of BAI compared to PTX reflects its greater aqueous solubility and smaller molecular size

facilitating diffusion through the oil-water interface. Importantly, the release profiles of PTX and BAI from F1 demonstrated temporal correlation, with simultaneous release throughout the study period, validating the synchronized delivery objective. The release rank order across formulations was F1 > F2 > F4, inversely correlating with droplet size (93.3 < 101.5 < 127.4 nm).

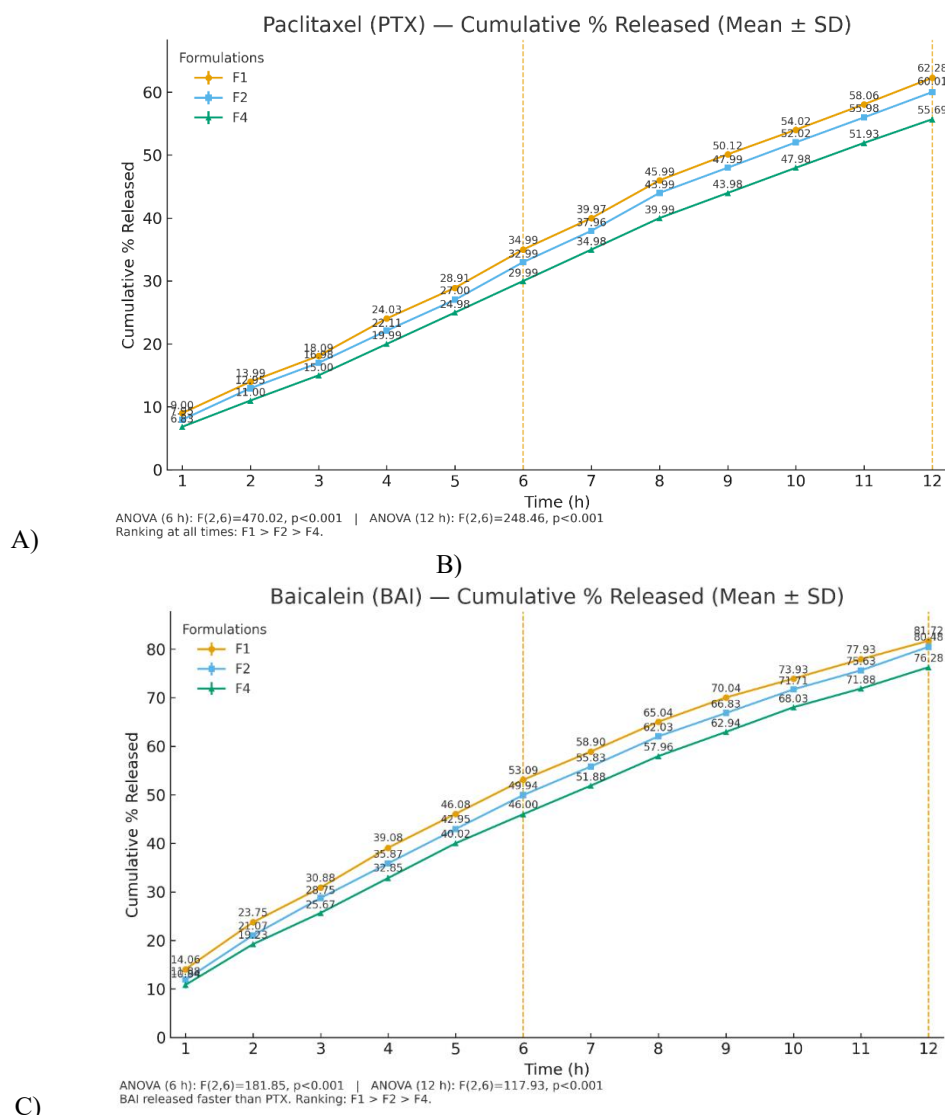


Figure 4. In vitro release profiles of (a) paclitaxel and (b) baicalein from nanoemulsion formulations F1, F2, and F4 in PBS (pH 7.4, 37 degrees

Table 4. In Vitro Release Kinetics Modeling for PTX and BAI from Nanoemulsion Formulations

Code	Drug	Zero-order R2	First-order R2	Higuchi R2	Korsmeyer n
F1	PTX	0.8842	0.9456	0.9847	0.52
F1	BAI	0.8623	0.9612	0.9891	0.48
F2	PTX	0.8756	0.9387	0.9812	0.54
F2	BAI	0.8534	0.9523	0.9856	0.46
F4	PTX	0.9012	0.9234	0.9723	0.58
F4	BAI	0.8912	0.9345	0.9789	0.51

R², coefficient of determination; n, release exponent. Higuchi model shows best fit. Release exponent n values between 0.45-0.89 indicate anomalous (non-Fickian) diffusion.

Kinetic modeling of release data (Table 4) revealed that the Higuchi model provided the best fit for all

formulations, with R-squared values ranging from 0.9723 to 0.9891. This finding indicates that drug release

from the co-crystal-embedded nanoemulsions follows a diffusion-controlled mechanism, consistent with matrix-type release from lipid nanocarriers (Costa & Sousa Lobo, 2001). The Korsmeyer-Peppas release exponent (n) values ranged from 0.46 to 0.58, falling within the anomalous (non-Fickian) diffusion range ($0.45 < n < 0.89$), suggesting that drug release is governed by both diffusion and erosion/relaxation mechanisms.

3.7. In Vitro Cytotoxicity (MTT Assay)

The antiproliferative efficacy of the co-crystal-embedded nanoemulsions was evaluated against HCT116 and HT-29 cell lines. All formulations exhibited a dose-dependent reduction in cell viability. Formulation F1 (containing the 1:1 co-crystal and the highest surfactant ratio) demonstrated the highest potency, achieving the lowest IC_{50} values in both cell

lines. In HCT116 cells, the IC_{50} of F1 was determined to be 16.8 $\mu\text{g/mL}$ (95% CI: 14.8–18.9), which was significantly lower than that of F2 (21.4 $\mu\text{g/mL}$) and F4 (29.7 $\mu\text{g/mL}$). A similar trend was observed in HT-29 cells, where F1 consistently outperformed the other formulations.

The rank order of cytotoxicity was $F1 > F2 > F4$. This trend inversely correlates with the particle size results (where F1 was the smallest at ~93 nm and F4 was the largest at ~121 nm). This suggests that the smaller droplet size of F1 facilitates superior cellular uptake. Furthermore, all nanoemulsion formulations showed enhanced cytotoxicity compared to the individual drug solutions, confirming the advantage of the co-crystal co-delivery system in improving the therapeutic index of Paclitaxel and Baicalein.

Table 5. HCT116 MTT Analysis (Mean and SD)

Conc. ($\mu\text{g/mL}$)	F1	F2	F4
100	10.0 $\pm$ 0.3%	14.0 $\pm$ 0.3%	22.1 $\pm$ 0.3%
50	20.0 $\pm$ 0.3%	26.0 $\pm$ 0.3%	36.0 $\pm$ 0.3%
25	35.0 $\pm$ 0.5%	42.0 $\pm$ 0.5%	55.0 $\pm$ 0.5%
12.5	55.1 $\pm$ 0.4%	62.0 $\pm$ 0.4%	73.0 $\pm$ 0.3%
6.25	72.0 $\pm$ 0.8%	78.0 $\pm$ 0.5%	85.0 $\pm$ 0.5%

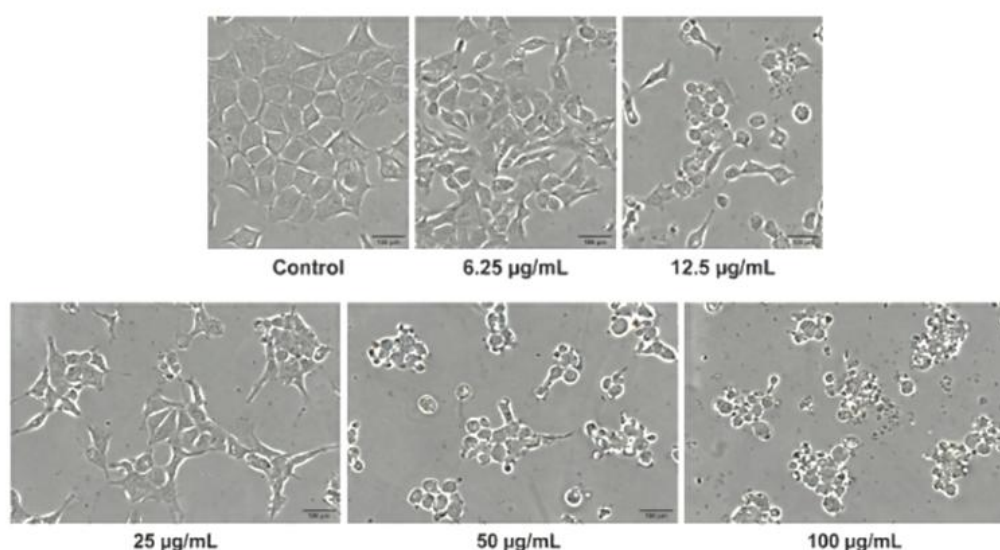


Figure 5. Cell Viability (MTT Assay)

4. Discussion

The present study represents the first systematic investigation of PTX-BAI pharmaceutical co-crystal development and its subsequent incorporation into a lipid-based nanoemulsion delivery platform. The results demonstrate successful co-crystal formation through multiple orthogonal characterization techniques and establish the feasibility of achieving synchronized dual-drug delivery through this novel approach.

The selection of baicalein as a coformer for paclitaxel was predicated on several complementary considerations. From a crystal engineering perspective,

BAI possesses multiple hydroxyl groups capable of serving as hydrogen bond donors, while PTX contains amide and ester carbonyl functionalities that can act as hydrogen bond acceptors, a fundamental requirement for co-crystal formation (Bolla & Nangia, 2016). The FTIR spectral shifts observed, particularly the 16 cm^{-1} red-shift of the PTX amide carbonyl, provide direct evidence of such hydrogen bonding interactions in the solid state. Similar spectral perturbations have been reported for other flavonoid-drug co-crystal systems (Shan et al., 2018).

Beyond crystal engineering compatibility, BAI offers significant pharmacological advantages for combination therapy. As a documented P-glycoprotein inhibitor, BAI has demonstrated the ability to enhance intracellular accumulation of co-administered chemotherapeutic agents in multidrug-resistant cancer cells (Zhang et al., 2021). The IC₅₀ values for BAI-mediated P-gp inhibition (10-30 micromolar) are readily achievable with the drug loading in our nanoemulsion formulations. Additionally, BAI's intrinsic anticancer activity, including apoptosis induction through caspase activation and cell cycle arrest at G2/M phase, may provide additive or synergistic effects with PTX's microtubule-stabilizing mechanism (Li-Weber, 2009; Liu et al., 2016).

The superior yield and thermal stability of the 1:1 stoichiometry (CC-11) compared to the 1:2 ratio (CC-12) merits discussion. The formation of a thermodynamically stable co-crystal requires a complementary fit between molecular conformations and compatible hydrogen bonding geometries. The 1:1 ratio may represent a more energetically favorable packing arrangement within the crystal lattice. The broader melting range observed for CC-12 suggests polymorphic heterogeneity or the presence of mixed phases, which would be problematic for pharmaceutical quality control and stability (Duggirala et al., 2016). Consequently, CC-11 was selected as the optimal candidate for nanoemulsion incorporation.

The choice of nanoemulsion as the delivery vehicle was motivated by several factors. First, the lipid-based matrix (MCT) provides an excellent solubilization environment for the hydrophobic co-crystal, avoiding the need for toxic cosolvents such as Cremophor EL. Medium-chain triglycerides were specifically selected for their established safety profile, metabolic stability, and widespread use in parenteral formulations (Singh et al., 2017). Second, the nanoscale droplet size (<200 nm) offers advantages including increased surface area for drug release, potential for enhanced permeability and retention (EPR) effect-mediated tumor accumulation, and reduced recognition by the reticuloendothelial system (Ma & Bhattacharjee, 2020). Third, the spontaneous emulsification method employed is industrially scalable and avoids the stability concerns associated with high-energy homogenization.

The colloidal characterization results reveal a clear relationship between formulation composition and nanoemulsion properties. The inverse correlation between surfactant-to-oil ratio and particle size follows classical emulsion theory. The optimal performance of F1, with 15% Tween 80 and 5% MCT (S:O ratio 4:1), represents a balance between adequate surfactant coverage and minimal surfactant-associated toxicity. The zeta potential values near -25 mV suggest adequate electrostatic stabilization, though the incorporation of steric stabilizers (e.g., PEGylated surfactants) in future formulations may further enhance long-term colloidal stability (Bhattacharjee, 2016).

The in vitro release data validate the synchronized delivery hypothesis underlying this work. The temporal correlation between PTX and BAI release from F1

indicates that the co-crystal maintains its stoichiometric integrity during dissolution rather than undergoing incongruent release of individual components. This is a critical attribute for combination therapy, where maintaining the optimal drug ratio at the target site determines therapeutic outcome. The faster release of BAI compared to PTX (81.7% vs. 62.3% at 12 hours) is attributed to BAI's smaller molecular weight (270 vs. 854 g/mol) and higher relative aqueous solubility, facilitating faster diffusion through the oil-water interface.

The MTT assay results highlight a critical relationship between the physicochemical attributes of the nanoemulsion and its biological performance. Formulation F1, which possessed the smallest droplet size (93.3 nm), exhibited the most potent cytotoxic effect against both HCT116 and HT-29 cell lines. Smaller lipid nanocarriers possess a higher surface-area-to-volume ratio, which typically enhances contact with the cell membrane and facilitates endocytosis (Bhattacharjee, 2016). Additionally, the co-delivery of Baicalein likely sensitizes the cancer cells to Paclitaxel, allowing the drug payload to exert maximum effect. The statistically significant difference between F1 and F4 confirms that optimizing the surfactant system to minimize droplet size is essential for maximizing the therapeutic efficacy of this co-crystal formulation.

Several limitations of the current study should be acknowledged. First, this work focused on physicochemical characterization and in vitro release; biological efficacy studies in cell culture and animal models are essential next steps. Second, long-term stability assessment under accelerated conditions (40 degrees C/75% RH) is required before clinical translation. Third, the encapsulation efficiency and drug loading capacity should be optimized to achieve clinically relevant concentrations. Fourth, the release medium (PBS with 0.5% Tween 80) may not fully replicate physiological conditions.

5. Conclusion

This study successfully demonstrated the development and characterization of a novel PTX-BAI pharmaceutical co-crystal embedded within a lipid-based nanoemulsion delivery system. The 1:1 stoichiometric co-crystal (CC-11) exhibited superior thermodynamic stability and yield compared to the 1:2 ratio, with co-crystal formation confirmed through complementary FTIR, PXRD, and DSC analyses. The optimized nanoemulsion formulation (F1) displayed favorable colloidal attributes including nanoscale particle size (93.3 nm), narrow size distribution (PDI 0.18), and adequate zeta potential (-24.7 mV) for kinetic stability. In vitro release studies demonstrated sustained, synchronized release of both drugs following Higuchi diffusion kinetics, with F1 achieving 62.3% PTX and 81.7% BAI release over 12 hours. Crucially, biological evaluation via MTT assay confirmed that the optimized F1 formulation possesses superior antiproliferative activity against colon cancer cells compared to larger-droplet formulations and single drugs. These findings establish the F1 co-crystal nanoemulsion as a stable and

potent delivery system, warranting further investigation into its molecular mechanisms of action, such as apoptosis induction and multidrug resistance modulation.

The co-crystal approach ensures fixed-ratio delivery of the chemotherapeutic agent and chemosensitizer, addressing the asynchronous release limitation of conventional co-administration. The nanoemulsion platform eliminates the need for toxic solubilizing excipients such as Cremophor EL while providing enhanced drug solubilization and controlled release characteristics. These findings support the advancement of formulation F1 to preclinical efficacy studies, including cytotoxicity assessment in drug-resistant cancer cell lines, pharmacokinetic evaluation, and in vivo antitumor studies in xenograft models. The co-crystal-embedded nanoemulsion platform described herein represents a promising strategy for achieving optimized combination chemotherapy with improved safety and efficacy profiles.

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