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Original Article

Development and Optimization of a Super Saturable Self-Nanoemulsifying Drug Delivery System for Ibrutinib: Enhancing Solubility and Anticancer Potential

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ABSTRACT

Aim: This study explores the development and optimization of a Super saturable Self-Nanoemulsifying Drug Delivery System to enhance the solubility, bioavailability, and anticancer efficacy of ibrutinib. **Materials and Methods:** Saturation solubility studies identified Kolliphor® RH 40, castor oil, and PEG-600 as the optimal surfactant, oil, and co-surfactant, respectively. Formulation optimization was performed using a Box-Behnken design, with droplet size and encapsulation efficiency as key response variables. Characterization included particle size analysis, zeta potential, FTIR, DSC, and XRPD studies, while *in vitro* drug release was assessed through diffusion studies. The MTT assay on MCF-7 and PANC-1 cell lines evaluated the cytotoxicity and therapeutic potential of the formulation. **Results:** The optimized S-SNEDDS for Ibrutinib demonstrated favorable characteristics, including a small droplet size (71.12-76.38 nm), high encapsulation efficiency (61.56-87.22%), and stability under stress conditions. The formulation exhibited rapid drug release, with over 50% of Ibrutinib released within 240 min. Characterization studies, including FTIR and DSC, confirmed the amorphous nature of the encapsulated drug. Kinetic studies suggested a zero-order drug release mechanism. Cytotoxicity evaluation via MTT assay indicated that the nanoformulation had lower cytotoxicity on MCF-7 and PANC-1 cells compared to pure Ibrutinib. **Conclusion:** These findings underscore the potential of S-SNEDDS as a promising approach to enhance the oral bioavailability of poorly water-soluble drugs like ibrutinib.

Keywords: Super saturable SNEDDS, Ibrutinib, Box-Behnken design, Nanocarriers, Drug solubility, Cytotoxicity, Cell lines.

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INTRODUCTION

Despite significant medical advancements, cancer remains a formidable challenge, with effective treatments still elusive. As a cornerstone of cancer treatment, chemotherapy encounters significant challenges both *in vitro* and *in vivo*, including the complex tumor microenvironment and Multidrug Resistance (MDR). To enhance therapeutic outcomes and minimize systemic toxicity, chemotherapy modulates multiple cell-signalling pathways.[1,2] Recently, nanocarrier-based drug delivery has emerged as a promising approach, improving solubility, stability, and controlled drug release. By facilitating targeted delivery of chemotherapeutic agents, these advanced technologies enhance drug exposure, efficacy, and safety, marking a transformative shift in cancer treatment paradigms.[3,4]

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Ibrutinib is a selective covalent inhibitor of Bruton's Tyrosine Kinase (BTK) and is extensively used for treating B-cell malignancies. As a small, orally active molecule, it irreversibly binds to the Cysteine Residue (C481) at BTK's phosphorylation site, effectively inhibiting the proliferation and division of malignant B cells.[5,6] However, ibrutinib exhibits poor aqueous solubility, dissolving weakly at pH 1.2 and becoming almost insoluble in the pH range of 3 to 8, which significantly limits its oral anticancer efficacy.[7] Additionally, its formulation is challenged by precipitation as it transitions from the stomach to the intestine due to pH variations.[8] To enhance its absorption and bioavailability, various formulation strategies have been explored, including self-emulsifying solid dispersions, lipid-based delivery systems, polymeric nanoparticles, nanocrystals, co-crystals, liquisolid technologies, and P-efflux inhibition.[9] These advanced approaches hold the potential to optimize ibrutinib delivery,

improving therapeutic effectiveness while enhancing patient compliance.

Among various strategies, Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) are widely recognized for their commercial viability, scalability, and effectiveness in enhancing oral drug delivery. Upon administration, drug-loaded SNEDDS spontaneously emulsify in Gastrointestinal (GI) fluids, forming oil plumes stabilized by surfactants and micelles with droplet sizes below 100 nm. These micellar structures enhance drug solubility, protecting it from GI degradation and pre-systemic metabolism.[10-13] The chylomicron-assisted intestinal absorption mechanism enables encapsulated drugs to bypass the central compartment by draining into lymphatic capillaries, effectively circumventing hepatic first-pass metabolism and maximizing oral bioavailability. SNEDDS exhibit remarkable thermodynamic and kinetic stability, forming an oil-in-water nanoemulsion within the GI tract's aqueous environment. This enhances drug absorption across gastrointestinal membranes, ultimately improving bioavailability. Beyond improving solubility and dissolution, SNEDDS have been widely explored for their impact on drug permeability, hepatic first-pass metabolism, and P-glycoprotein efflux, reinforcing their potential as an advanced oral drug delivery platform.[14-16]

The Quality by Design (QbD) approach systematically evaluates the multifactorial aspects of product development by incorporating design of experiments, risk assessment, principal component analysis, and process analytical technology. Increasingly adopted by scientists, QbD aims to streamline processes, reduce costs, and enhance product quality within defined timelines. In line with this framework, this study employed a QbD-driven strategy to develop an ibrutinib-loaded SNEDDS, ensuring an optimized formulation.[17,18]

One major challenge with SNEDDS formulations for poorly soluble drugs is precipitation in gastric media, which compromises both solubility and bioavailability. While surfactants are commonly used to mitigate this issue, excessive surfactant levels can cause GI irritation and toxicity.[19,20] A super saturable Self-Nanoemulsifying Drug Delivery System (S-SNEDDS) was formulated to overcome these challenges. This advanced formulation integrates a water-soluble polymeric precipitation inhibitor, which helps maintain a meta-stable supersaturated state by preventing drug precipitation upon aqueous dilution. By stabilizing the drug in a solubilized form, S-SNEDDS enhances intestinal absorption, ensuring higher drug permeability across the gastrointestinal membrane. This strategy significantly improves the oral bioavailability of poorly soluble drugs like ibrutinib, potentially leading to better therapeutic outcomes and more consistent plasma drug concentrations.[21-25]

To optimize the formulation, Response Surface Methodology (RSM)-specifically the Box-Behnken Design (BBD)-was employed to assess how independent formulation variables influence key response parameters. Ibrutinib-loaded SNEDDS were formulated with GRAS (Generally Recognized as Safe) excipients and systematically optimized through a 3-level, 3-factor BBD. The optimized formulation underwent comprehensive physicochemical characterization and stability testing, reinforcing the precision and effectiveness of this QbD-driven formulation strategy for enhanced oral delivery of ibrutinib.

MATERIALS AND METHODS

Materials

Ibrutinib was sourced from Aspen Biopharma Labs Pvt. Ltd., Hyderabad. Excipients, including surfactants, co-surfactants, and oils, were obtained from SD Fine Chemicals, Mumbai, India. To maintain the integrity and reproducibility of the experiments, the study exclusively utilized Millipore Milli-Q water. Additionally, only analytical-grade chemicals and reagents were employed to ensure accuracy and reliability in formulation development and characterization.

Development of SNEDDS

Saturation Solubility and Emulsification Efficiency Studies for Component Selection

A saturation solubility study was performed using the shake flask method to identify the optimal oil, surfactant, and co-surfactant for ibrutinib formulation.[26] Various oils, including Linseed, Olive, Castor, Sesame, Groundnut, and Soybean oil, along with surfactants and co-surfactants such as Kolliphor® EL, Kolliphor® RH 40, Tween®, Span®, Carbitol, Triton X-100, Polyethylene Glycols (PEGs), Propylene glycol, and Ethanol, were evaluated. Ibrutinib was combined with 1 g of each vehicle and vortexed to ensure uniform mixing. The samples were continuously agitated in a water bath shaker at 100 rpm and maintained at 37°C for a duration of 72 hr. Following incubation, the samples were centrifuged at 10,000 rpm for 15 min, and the resulting supernatant was diluted with methanol. The concentration of ibrutinib was then determined through UV spectrophotometric analysis. To select the optimal surfactant, 100 mg of each surfactant was mixed with 100 mg of the chosen oil, vortexed, and diluted with 25 mL of distilled water to form an emulsion. The emulsification efficiency was assessed based on the number of inversions required for uniformity, and transmittance was measured at 638.2 nm after standing for 2 hr. Similarly, co-surfactants were screened by combining 100 mg of surfactant with 50 mg of co-surfactant (2:1 ratio), followed by the addition of 150 mg of oil, vortexing, and dilution with 25 mL of double-distilled water. Emulsification efficiency was evaluated using inversion count and transmittance measurement at 638.2 nm.

Nanoemulsion Region Identification - Ternary phase diagram

To determine the nanoemulsion region, a pseudo ternary phase diagram was developed using a modified visual test method by Craig *et al*.[27,28] The formulations were designed with oil (40-80% w/w) for drug solubilization and stability, surfactant (20-60% w/w) to facilitate emulsification and reduce interfacial tension, and co-surfactant (0-20% w/w) to enhance nanoemulsion formation, ensuring a total composition of 100% w/w for an optimized self-nanoemulsifying drug delivery system. Each mixture was vortexed for 60 seconds to achieve uniform blending, then diluted (100 mg in 25 mL double-distilled water) and gently agitated to assess nanoemulsion formation. Only formulations producing clear or slightly bluish dispersions with droplet sizes below 200 nm were considered part of the nanoemulsion region. The study was performed three times to confirm the consistency and reliability of the results.

Experimental Design-Based Optimization of SNEDDS

A 3^3 BBD was used to systematically optimize the liquid SNEDDS formulation, ensuring a robust and well-characterized drug delivery system. This statistical approach efficiently models quadratic response surfaces, allowing for a detailed examination of primary influences, interactive relationships, and nonlinear effects of formulation variables. The design included 17 experimental runs, incorporating five center-point replicates to enhance the reliability of the optimization process (Table 1). Castor oil (A), Kolliphor® RH 40 (B), and PEG-600 (C) were chosen as independent variables, each adjusted at three levels-low (-1), middle (0), and high (+1) - to investigate their impact on critical formulation attributes. The primary response factors-droplet size (Y1) and encapsulation efficiency (Y2)-were analyzed using Design Expert® software was used to construct a second-order quadratic model, providing a mathematical representation of the relationship between independent and dependent variables.[29,30]

To ensure precision, backward elimination was used to refine the quadratic model by systematically removing non-significant terms. Model adequacy was evaluated using Analysis of Variance (ANOVA), lack-of-fit tests, and regression analysis, incorporating the coefficient of determination (R^2) to verify the reliability and predictive precision of the quadratic model. Coefficients were interpreted based on their impact on the response variables, where positive values indicated synergistic effects, while negative values suggested antagonistic interactions. Coefficients with p-values less than 0.05 were considered statistically significant. The selected model was evaluated using perturbation plots and three-dimensional (3D) response surface plots, offering a comprehensive visualization of formulation dynamics.

To balance multiple responses, a desirability function was employed, utilizing Derringer and Such's numerical optimization method in Design Expert software. This method assigned each response a desirability score between 0 and 1, where 0 indicated an undesirable outcome and 1 represented the most favorable result. This approach enabled the identification of an optimal SNEDDS formulation, balancing droplet size and encapsulation efficiency, ensuring enhanced drug solubility, stability, and bioavailability.[31]

SNEDDS preparation

The ibrutinib loaded SNEDDS was formulated by dissolving a specified amount of ibrutinib in a carefully optimized mixture of oil, surfactant, and co-surfactant. To achieve uniform dissolution, the mixture was subjected to stirring, vortexing, and controlled heating at 37ºC using a magnetic stirrer. After complete dissolution, the formulation was kept at room temperature for subsequent analysis. Similarly, the placebo SNEDDS (without ibrutinib) was formulated using the same optimized composition and preparation techniques. The thorough blending and homogenization of ingredients ensured the development of a well-structured and stable placebo formulation, essential for comparative studies.

Development of supersaturable SNEDDS

Selection of Precipitation Inhibitor and Preparation of Super saturable SNEDDS

To ensure stable supersaturation and mitigate drug precipitation, *in vitro* precipitation studies were conducted using various polymeric precipitation inhibitors, including Hydroxypropyl Methylcellulose (HPMC) K4M, Polyvinylpyrrolidone (PVP) K30, Poloxamer-407, and Eudragit® L100. For the study, a 100 mg sample of the optimized SNEDDS formulation containing the selected polymer was dispersed in 100 mL of simulated gastric fluid (SGF, pH 1.2) to replicate stomach conditions. The system was maintained at a constant temperature of 37±0.5ºC with continuous agitation at 100 rpm using a magnetic stirrer to mimic physiological conditions. At predetermined time intervals (5, 15, 30, 45, 60, 90, 120, 180, and 240 minutes), 1 mL aliquots were withdrawn without replenishing the volume to prevent dilution effects. These samples were immediately centrifuged at 12,000 × g for 5 min to separate any precipitated drug. The resulting supernatant was carefully collected and diluted with methanol to dissolve any residual drug before being analyzed using UV spectrophotometry to quantify the ibrutinib concentration over time.

To formulate the super saturable Self-Nanoemulsifying Drug Delivery System (S-SNEDDS), 5% w/w of the selected precipitation inhibitor was incorporated into the optimized SNEDDS using a simple yet effective admixture technique. The formulation was vortexed vigorously for 5 min to ensure complete dispersion of the polymer and achieve homogeneity. Following this, the mixture was placed in a controlled water bath set at 37±0.5ºC and equilibrated for 24 hr.

Physicochemical Characterization of Ibrutinib SNEDDS and S-SNEDDS

The physicochemical characteristics of ibrutinib and its SNEDDS and S-SNEDDS formulations were extensively evaluated using various analytical techniques to ensure stability, efficacy, and optimal performance.

Size distribution and zeta potential

Droplet size and zeta potential were analyzed to assess the stability and uniformity of SNEDDS formulations. Each sample was diluted 100-fold with deionized water, gently agitated, and measured using Dynamic Light Scattering (DLS) with a Mastersizer 2000 (Malvern Instruments, UK) at a fixed angle of 90°. Zeta potential, indicating colloidal stability, was determined using an integrated electrode in the same instrument. Measurements were conducted in triplicate, and the mean droplet size and Polydispersity Index (PDI) were calculated to ensure accuracy and reliability.

Surface Morphology Analysis

The morphology of ibrutinib-loaded SNEDDS and S-SNEDDS was examined using a JEOL JEM 2100 F microscope (USA), Transmission Electron Microscopy (TEM). The formulations were diluted by a factor of 1000 using distilled water, carefully applied to a coated carbon grid, and subsequently stained with a 2% uranyl acetate solution. After thorough rinsing with distilled water, the samples were examined under 200 kV radiation with a 100 cm camera length, capturing high-resolution two-dimensional X-ray patterns to reveal nanoscale structural details.

Physicochemical Characterization: FTIR, DSC, and XRPD Analysis

FTIR spectroscopy, DSC, and XRPD analyses were conducted to assess chemical stability, thermal behavior, and structural characteristics of ibrutinib, SNEDDS, and S-SNEDDS.FTIR Spectra were recorded using the KBr disc method with a Schimadzu FTIR 8400S spectrophotometer (Japan) over a 400-4000 cm^{-1} range to detect potential interactions between formulation components and ensure chemical stability. Thermal behavior was analyzed using a Perkin Elmer DSC/7 calorimeter, calibrated with indium. A precisely weighed 5 mg sample was sealed in an aluminum pan and subjected to controlled heating from 30ºC to 400ºC at a uniform rate of 10ºC/min. An empty aluminum pan was used as a reference to establish baseline thermal changes. The study was conducted under a continuous nitrogen purge to prevent oxidative degradation and ensure consistent heat flow measurements. The structural properties of ibrutinib, SNEDDS, and S-SNEDDS were analyzed using a Bruker D8 Advance diffractometer to determine their crystalline or amorphous nature. The diffraction patterns were recorded at a scan rate of 5° per minute over a 2θ range of 10° to 80° under ambient conditions, offering valuable insights into the structural characteristics of the drug and its formulations.

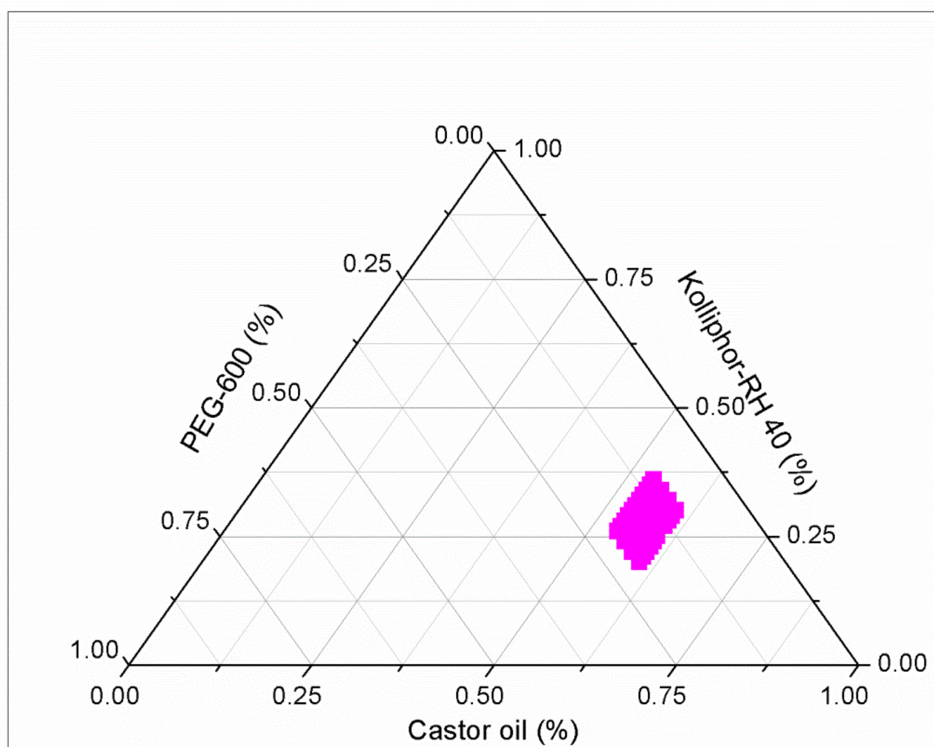

Figure 1: Ternary phase diagram of drug loaded SNEDDS.

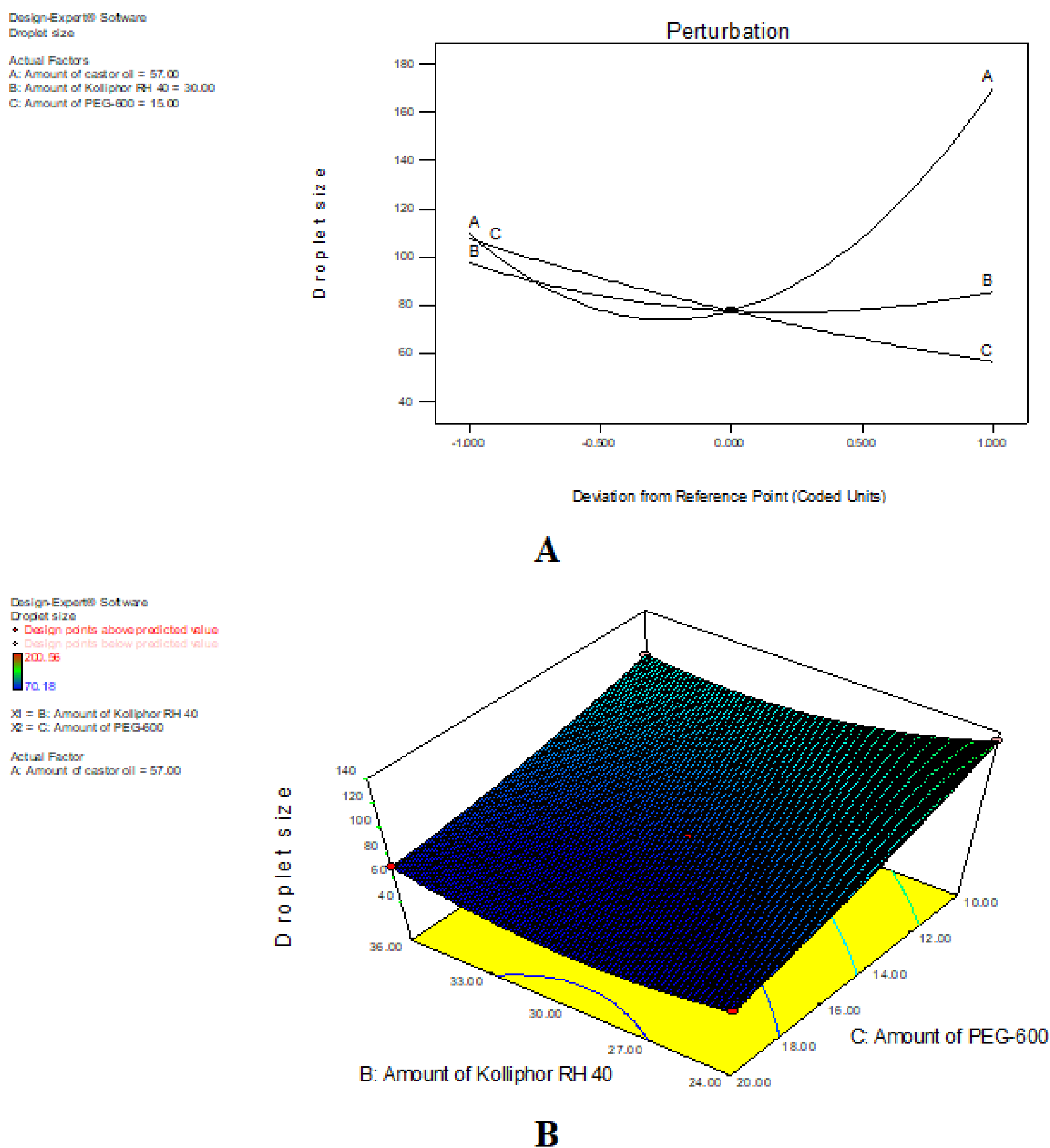

Figure 2: A. Themain effects of effects of A, B, C on Y1. B. The interactive effect of B and C on Y1.

Refractive Index Measurement

The isotropic nature of ibrutinib-loaded SNEDDS and S-SNEDDS was evaluated by measuring the Refractive Index (RI). Each formulation was diluted 1000-fold with distilled water, and a single drop was analyzed at room temperature using an Abbes-type Refractometer. To ensure precision, measurements were performed in triplicate, and the average values were recorded for a reliable evaluation of isotropic properties.

Evaluation of Optical Clarity and Transmittance (%)

The percentage transmittance (%T) of ibrutinib-loaded SNEDDS and S-SNEDDS, indicating optical clarity, was measured using a double-beam UV spectrophotometer at 650 nm. Distilled water was used as the blank. A 1000-fold dilution of each formulation was prepared using distilled water, then transferred to a stoppered volumetric flask and left undisturbed for 2 hours before measurement. To ensure an accurate assessment of formulation transparency, average values were recorded after repeating the procedure three times.

Determination of Drug Content in SNEDDS and S-SNEDDS

Spectrophotometric analysis at 260 nm was used to determine the drug content in SNEDDS and S-SNEDDS formulations. A precisely weighed sample was dissolved in 10 mL of methanol and vortexed for 10 min to achieve complete dissolution. The solution was then passed through a 0.45 μm membrane filter and analyzed against a blank reference for accuracy. The assay was conducted on three separate samples, and the mean values were recorded to ensure reliability and precision.

In vitro Dissolution and Drug Release Kinetics

The dissolution behavior of ibrutinib-loaded SNEDDS and S-SNEDDS was studied using a USP Type II apparatus (Electrolab, TD L8) under sink conditions. To simulate gastric conditions, 420 mg of the formulation was introduced into 500 mL of Simulated Gastric Fluid (SGF, pH 1.2) maintained at 37°C, with the paddle rotating at 50 rpm. At specific time intervals (5, 10, 15, 30, 45, 60, 90, and 120 min), 5 mL samples were withdrawn and immediately replaced with fresh medium to maintain a constant volume. After 2 hr, the dissolution medium was adjusted to pH 6.8 by adding 250 mL of 0.3 M dibasic sodium phosphate, mimicking intestinal conditions (Simulated Intestinal Fluid - SIF). The collected samples were filtered, appropriately diluted, and analyzed via UV spectrophotometry to determine drug concentration. To ensure reliability, the study was conducted in triplicate. Furthermore, drug release kinetics were evaluated using zero-order, first-order, Higuchi, and Korsmeyer-Peppas models to characterize the release mechanism.

Stability Evaluation of SNEDDS and S-SNEDDS

Thermodynamic Stability

To assess thermodynamic stability, the formulations were exposed to rigorous stress conditions, including six alternating series of cooling (4°C) and heating (40°C), trailed by freeze-thaw cycles ranging from -21°C to 25°C over a 48-hr period. Additionally, centrifugation at 3500 rpm for 30 min was performed to detect any phase separation. Formulations that rapidly reverted to their original state within 2-3 min were classified as thermodynamically stable and considered suitable for further investigation.

Stability in Simulated GI Fluids

To replicate gastrointestinal conditions, 500 mg of SNEDDS was first immersed in 50 mL of Simulated Gastric Fluid (SGF, pH 1.2) for 2 hr, then transferred to Simulated Intestinal Fluid (SIF, pH 6.8) for an additional 6 hr. After incubation, droplet size and Polydispersity Index (PDI) were measured to assess formulation stability and evaluate the likelihood of drug precipitation.

Dilution Stability

To assess the stability of ibrutinib-loaded SNEDDS under varying dilution conditions, the formulation was diluted 200-, 400-, 600-, and 800-fold in Simulated Gastric Fluid (SGF, pH 1.2), incubated at 37°C for 2 hr, and analyzed using a Mastersizer 2000 (Malvern, UK) to determine droplet size and Polydispersity Index (PDI).

Accelerated Stability Testing

An accelerated stability study was conducted in accordance with ICH Q1A (R2) guidelines to simulate long-term storage conditions and evaluate the stability of the formulations. The samples were stored in a controlled stability chamber maintained at 40°C and 75% relative humidity for a period of three months. Throughout the study, critical parameters such as droplet size, zeta potential, and Polydispersity Index (PDI) were systematically analyzed both before and after storage. Any deviations in these parameters were meticulously documented to assess the formulations' physicochemical stability, structural integrity, and overall resilience under elevated temperature and humidity conditions.

Cytotoxicity and Antiproliferative Assessment Using MTT Assay

To evaluate the cytotoxicity and antiproliferative effects of the Ibrutinib test formulation, an MTT assay was conducted using PANC-1 and MCF-7 cell lines. A total of 2×10^4 cells per well were carefully seeded into 96-well plates, each containing 200 µL of fresh culture medium, to ensure uniform cell distribution. The plates were then incubated under controlled conditions at 37±1°C with 5±1% CO_2 for 24 hr, allowing the cells to adhere and reach optimal confluency before further experimental treatments. Following the initial incubation, the cells were treated with the Ibrutinib test formulation and kept under identical conditions for an additional 48 hr. Thereafter, 100 µL of MTT solution (0.5 mg/mL) was added to each well and incubated for 3 hr to assess cell viability. The resulting formazan crystals were dissolved, and absorbance was measured at 570 nm using a spectrophotometer. Untreated cells served as the negative control (100% viability), and treated groups were compared against this reference. Dose-response data were fitted using a Four-Parameter Logistic (4PL) regression model to calculate the half-maximal Inhibitory Concentration (IC_{50}) and maximum Inhibitory effect (I_{max}).

Table 1: BBD with experimental results.

Run	A (mg)	B (mg)	C (mg)	Y1 (nm)	Y2 (%)
1	54	24	15	128.82	63.48
2	60	24	15	189.92	61.56
3	54	36	15	118.85	80.23
4	60	36	15	176.89	70.12
5	54	30	10	138.82	72.48
6	60	30	10	200.56	69.92
7	54	30	20	88.53	85.68
8	60	30	20	148.56	75.66
9	57	24	10	133.89	65.34
10	57	36	10	108.26	82.84
11	57	24	20	70.18	80.58
12	57	36	20	71.84	86.38
13	57	30	15	77.86	87.22
14	57	30	15	79.58	86.12
15	57	30	15	76.88	85.26
16	57	30	15	78.12	86.78
17	57	30	15	76.54	84.42

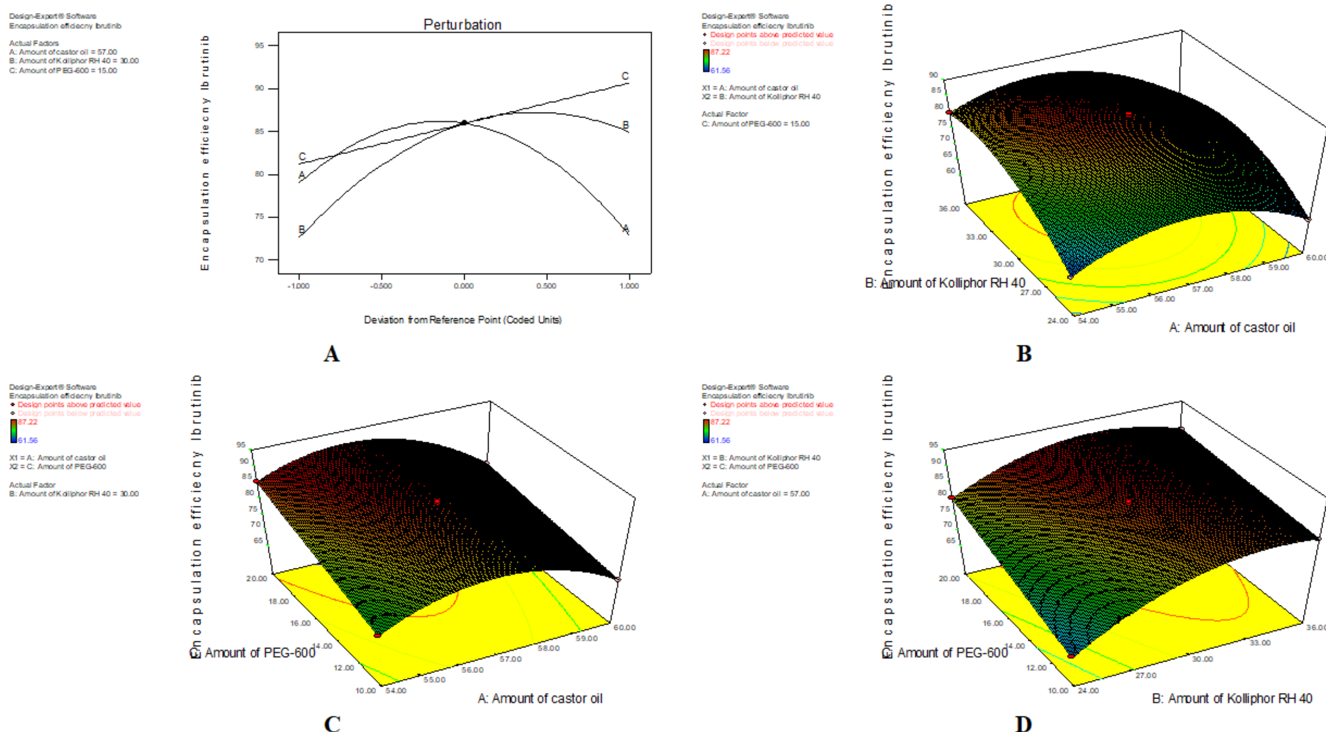

Figure 3: A. The main effects of A, B, and C on Y2. B. The interactive effect of A and B on Y2.

Nonlinear curve fitting and statistical analyses were performed using GraphPad Prism.[33] The viability of the cells was determined using Equation 1.

$$\%\,Viability = \frac{Absorbance\ of\ treated\ cellls}{Absorbance\ of\ control\ cellls} x\ 100\ (1)$$

Where:

Absorbance of treated cells refers to the Optical Density (OD) measured at 570 nm for cells exposed to the test formulation.

Absorbance of control cells refers to the OD measured at 570 nm for untreated cells (negative control), which is considered 100% viable.

Statistical evaluation

All data were presented as mean±SD, and statistical analyses were conducted to evaluate variations among different formulations. One-way ANOVA was employed to assess overall differences, while Student's *t*-test was utilized for pairwise comparisons between specific groups. A significance level of $p<0.05$ was established to identify statistically significant differences, ensuring the reliability and validity of the results.

RESULTS

Solubility Studies and Selection of Excipients

The selection of suitable components for SNEDDS formulation was guided by evaluating ibrutinib's solubility across different oils, surfactants, and co-surfactants to determine the most effective solubilizing agents. Among the tested oils, Castor oil demonstrated the highest ability to dissolve ibrutinib, with a solubility of 3456.28±216.78 µg/mL, while Linseed oil exhibited the lowest solubility at 982.58±48.78 µg/mL. Due to its exceptional solubilization efficiency, Castor oil was chosen as the preferred oil phase for further formulation optimization. Kolliphor® RH 40 (10123.56±342.43 µg/mL), Kolliphor® EL (6992.56±298.82 µg/mL), Tween-80 (6378.12±432.15 µg/mL), and PEG-600 (5768.32±198.67 µg/mL) exhibited the highest solubilization capacities among the tested surfactants and co-surfactants. Seven non-ionic surfactants-Kolliphor® RH 40, Kolliphor® EL, Tween-80, Span-80, Tween-20, Span-20, and Carbitol-were assessed for their emulsification efficiency and percent Transmittance (%T) to determine their suitability for formulation. Among them, Kolliphor® RH 40 demonstrated the highest emulsification performance with castor oil, making it the preferred surfactant. Five co-surfactants, including Triton™ X-100, PEG 600, PEG 400, PEG 300, and Propylene glycol, were tested with Kolliphor RH 40 in a fixed 1:1 ratio to assess their compatibility and performance in the formulation. PEG-600 provided optimal emulsification, higher % transmittance, and ease of emulsification, making it the preferred co-surfactant.

Construction of Pseudo-Ternary Phase Diagram

A ternary phase diagram (Figure 1) was developed to optimize the ratio of oil, surfactant, and co-surfactant, ensuring the

formation of a stable and effective nanoemulsion. Various combinations were systematically tested, and the region exhibiting spontaneous nanoemulsification with clear or slightly bluish dispersions was identified. This optimized ratio ensured effective solubilization of ibrutinib while maintaining formulation stability and self-emulsifying properties. Formulations within this region produced droplet sizes <200 nm, ensuring efficient self-emulsification and enhanced drug absorption. In the ternary phase diagram, the highlighted region signifies the area where a clear and transparent oil-in-water emulsion is formed with minimal agitation. Meanwhile, the shaded sections represent zones with the highest probability of generating nanoemulsions characterized by droplet sizes under 200 nm, ensuring enhanced solubilization and stability. Increasing the ratio of Kolliphor® RH 40 to PEG-600 led to an expansion of the nanoemulsion region, suggesting enhanced surfactant adsorption at the interface. This improvement reduced surface tension, ultimately promoting the formation of smaller droplet sizes. The pink-shaded region in the diagram represents the optimal self-nanoemulsifying zone, characterized by the formation of uniform, transparent dispersions when diluted with water. Based on the analysis, the optimal formulation range for each component was established as Castor oil (54%-60%), Kolliphor® RH 40 (24%-36%), and PEG-600 (10%-20%).

Optimization of Formulation Using Box-Behnken Design

To optimize SNEDDS, a Box-Behnken design was implemented, allowing for a systematic evaluation of formulation variables, including their individual impact, interactions, and quadratic influences. The selected independent variables were: Castor oil (A): 54-64 parts by weight; Kolliphor® RH 40 (B): 36-46 parts by weight and PEG-600 (C): 10-20 parts by weight. Seventeen experimental runs were designed and executed in a randomized sequence using the BBD model, generated through Stat-Ease Design Expert® software V13.0.5.0. Table 1 outlines the specific conditions for each experiment and the corresponding outcomes for droplet size (Y1) and encapsulation efficiency (Y2). The observed droplet sizes ranged from 70.18 to 200.56 nm, while encapsulation efficiency varied between 61.56% and 87.22%. The data were fitted to a second-order quadratic model, and its suitability was assessed using ANOVA, lack-of-fit tests, and R^2 analysis through Design-Expert software to ensure accuracy and reliability.[34,35] The quadratic model was deemed the most appropriate, as it demonstrated a high F-value and a non-significant lack-of-fit ($p>0.1$), confirming its robustness in predicting the response variables. The R^2 values further supported the model's reliability in explaining data variability. RSM successfully minimized the number of experimental runs by efficiently assessing the interaction effects between the formulation components. The data were further analyzed using ANOVA, regression coefficients, and 3D response surface plots to visualize the influence of each factor on SNEDDS performance.

Droplet size (Y1) is a critical factor in SNEDDS performance, influencing drug absorption and release rate. Smaller droplets provide a larger interfacial surface area, enhancing bioavailability. In this study, droplet size ranged from 70.18 to 200.56 nm. The

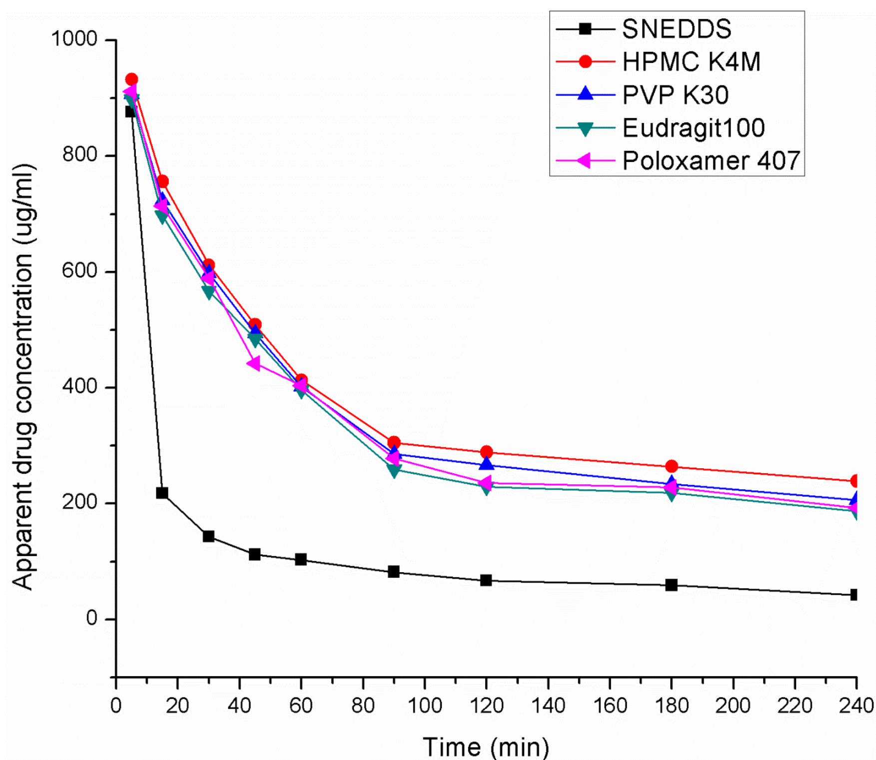

Figure 4: Effect of different polymers on *in vitro* mean concentration profiles of ibrutinib SNEDDS.

optimization of droplet size (Y1) using the quadratic model was statistically significant, as indicated by an F-value of 3796.51. This indicates that the likelihood of the observed results occurring by chance is merely 0.01%. Droplet size was significantly affected by the key model terms A, B, C, BC, A^2, B^2, and C^2 ($p<0.05$). The model's Lack of Fit F-value of 0.65 indicated no statistically significant deviation from the expected fit, which aligns with the expected criteria for a well-fitting model. The model's high predictive accuracy was demonstrated by Predicted R^2 (0.9990), closely matching Adjusted R^2 (0.9994) and Adequate Precision of 176.405, well above the desired threshold of 4. Equation 2 represents the regression model for droplet size (Y1) formulated based on the second-order quadratic equation. The perturbation plot (Figure 2A) revealed that Castor oil (A) had the greatest effect on droplet size, with Kolliphor® RH 40 (B) and PEG-600 (C) following in significance. Figure 2B illustrates that the interaction between B and C, while holding A constant, also significantly affected the droplet size.

$$Y1 = 77.80 + 30.11A - 5.87B - 25.30C + 6.82\mathrm{BC} + 61.95A^2 + 13.87B^2 + 4.37C^2 \quad (2)$$

The encapsulation efficiency (Y2) of ibrutinib-loaded SNEDDS, influenced by drug concentration, oil, surfactant, co-surfactant, and process variables, ranged from 61.56% to 87.22%. Statistical analysis confirmed a quadratic model as the best fit, with a high Model F-value of 225.39 and only a 0.01% likelihood that the results were influenced by noise. The encapsulation efficiency was significantly influenced by the key model terms (A, B, C, AB, AC, BC, A^2, and B^2), with p-values less than 0.05. The model's accuracy was validated by a Lack of Fit F-value of 0.11, indicating an insignificant lack of fit with a 97.35% likelihood of resulting from random noise. High predictive accuracy, with Predicted R^2 (0.9901) and Adjusted R^2 (0.9912) closely aligned. Adequate Precision (40.627), far exceeding the acceptable threshold of 4. Equation 3 represents the regression model for encapsulation efficiency (Y2) formulated based on the second-order quadratic equation. Observed values closely matched predicted values, confirming the model's reliability. The analysis also highlighted Kolliphor® RH 40 (B) as the most influential factor, followed by PEG-600 (C) and Castor oil (A) (Figure 3).

$$Y2 = 85.94 + 3.08A + 6.08B + 4.71C - 2.05\mathrm{AB} - 1.87\mathrm{AC} - 2.93\mathrm{BC} - 9.98A^2 - 7.13B^2 \quad (3)$$

The interactive effect of A and C on Y2.D. The interactive effect of B and C on Y2.

The desirability function approach was employed to optimize ibrutinib-loaded SNEDDS, balancing two key response variables. Each response was transformed into a desirability scale, where the highest (Y_{max}) and lowest (Y_{min}) values set the boundaries. The optimized formulation was obtained with 56.32% Castor oil (A), 31.32% Kolliphor® RH 40 (B), and 15.91% PEG-600 (C). This combination resulted in a maximum desirability value (D) of 1.000, ensuring an optimal balance between formulation components. To validate the model, three formulations were prepared under the optimized conditions, and their experimental responses closely matched predicted values, confirming the model's accuracy.

Preparation and Characterization of SNEDDS and S-SNEDDS

Screening for a Precipitation Inhibitor

The goal of supersaturable SNEDDS (S-SNEDDS) is to maintain a supersaturated state, preventing drug precipitation and enhancing bioavailability. Each polymer was added to the optimized ibrutinib SNEDDS (S1) at a concentration of 5% of the total formulation weight. The study used 100 mL of medium, simulating residual stomach fluid volume. The mean concentration-time profile of ibrutinib was monitored for 240 min to assess precipitation inhibition (Figure 4). The SNEDDS formulation without a precipitation inhibitor showed rapid precipitation, with ibrutinib concentration dropping to 218 µg/mL at 15 min and 143 µg/mL at 30 min. In contrast, S-SNEDDS formulations exhibited significantly higher ibrutinib concentrations, demonstrating superior precipitation inhibition. Among the tested inhibitors, HPMC K4M was the most effective, maintaining higher drug concentration levels throughout the study.[36,37]

The droplet size of SNEDDS formulations (S1-S3) ranged from 71.12 to 76.38 nm, while S-SNEDDS formulations (F1-F3) exhibited smaller sizes, ranging from 58.76 to 67.12 nm. The reduced droplet size in S-SNEDDS enhances drug absorption by

Table 2: Droplet size, PDI, and zeta potential measurements.

Sample	Average droplet size±SD (nm)	PDI	ZP (mV)
S1(SNEDDS)	75.34±4.24	0.193±0.005	-24.6 ±2.1
S2(SNEDDS)	76.38±5.26	0.126±0.005	-25.8 ±1.6
S3(SNEDDS)	71.12±3.14	0.312±0.005	-28.4 ±2.3
F1(S-SNEDDS)	58.76±1.76	0.097 ±0.005	-22.3±1.4
F2(S-SNEDDS)	63.12±2.04	0.212 ±0.005	-26.4±1.7
F3(S-SNEDDS)	67.12±1.33	0.064 ±0.005	-23.7±2.2

(All measurements were performed three times, and the results are expressed as mean±SD, with a sample size of n=3).

increasing the surface area. PDI values ranged between 0.124 and 0.312 indicate uniform size distribution across all formulations. Zeta Potential measured between -23.7 to -28.4 mV, suggesting strong electrostatic repulsion among droplets, contributing to colloidal stability. The detailed values are presented in Table 2.

TEM imaging (Figure 5) revealed that SNEDDS and S-SNEDDS formulations formed uniform, spherical nanoparticles, confirming the nanoscale size and structural integrity of the formulation. This uniformity is essential for enhanced stability and bioavailability.

FTIR analysis (Figure 6A) showed that the characteristic peaks of ibrutinib remained unchanged in SNEDDS and S-SNEDDS formulations, confirming the absence of any chemical interactions between the ibrutinib and excipients. The slight masking of peaks suggests successful drug encapsulation within the formulation. XRPD analysis (Figure 6B) revealed that pure ibrutinib exhibited a paracrystalline nature, while drug-loaded SNEDDS and S-SNEDDS showed complete amorphousness, confirming drug encapsulation and enhanced solubility. DSC thermograms (Figure 6C) demonstrated a sharp endothermic peak for pure ibrutinib, which was absent in SNEDDS and S-SNEDDS, further confirming the amorphous transformation of ibrutinib within the formulation.

The Refractive Index (RI) was measured to confirm the isotropic nature of S-SNEDDS nanoemulsions. All formulations maintained their isotropic properties after emulsification, with values ranging from 1.23 to 1.34, indicating uniform dispersion. The transmittance study further verified the isotropic nature of S-SNEDDS. All batches exhibited nearly 100% transmittance, confirming the clarity and homogeneity of the formulations.

In vitro Dissolution and Kinetic Modeling

The drug release characteristics of pure drug ibrutinib, SNEDDS, and S-SNEDDS were assessed in both Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) to evaluate the drug's release characteristics under different physiological conditions. SNEDDS formulations demonstrated significantly faster drug release compared to pure ibrutinib, attributed to rapid emulsification and a larger surface area of nano-sized droplets. S-SNEDDS formulations achieved even faster dissolution, releasing 43.5±1.8% of ibrutinib within 5 minutes, outperforming both pure drug dispersion and SNEDDS formulations (Figure 7). The improved dissolution resulted from the transformation of ibrutinib from its crystalline form into an amorphous or molecularly dispersed phase within S-SNEDDS.

The optimized SNEDDS and S-SNEDDS formulations were analyzed to explore the drug release kinetics. The release profiles of ibrutinib exhibited a consistent trend across all formulations, with regression coefficients nearing unity, especially in zero-order kinetics, suggesting a steady dissolution rate over time (Table 3). Mathematical modeling further confirmed that the drug release followed zero-order kinetics, indicating a consistent and controlled release profile. The exploration continued with Higuchi and Korsmeyer-Peppas plots, where the n value from Korsmeyer-Peppas indicated super case II transport, highlighting a more complex release mechanism beyond simple diffusion. These findings underscore the optimized SNEDDS formulation's role as not just a drug carrier but a refined system that reveals the complexities of drug release dynamics.

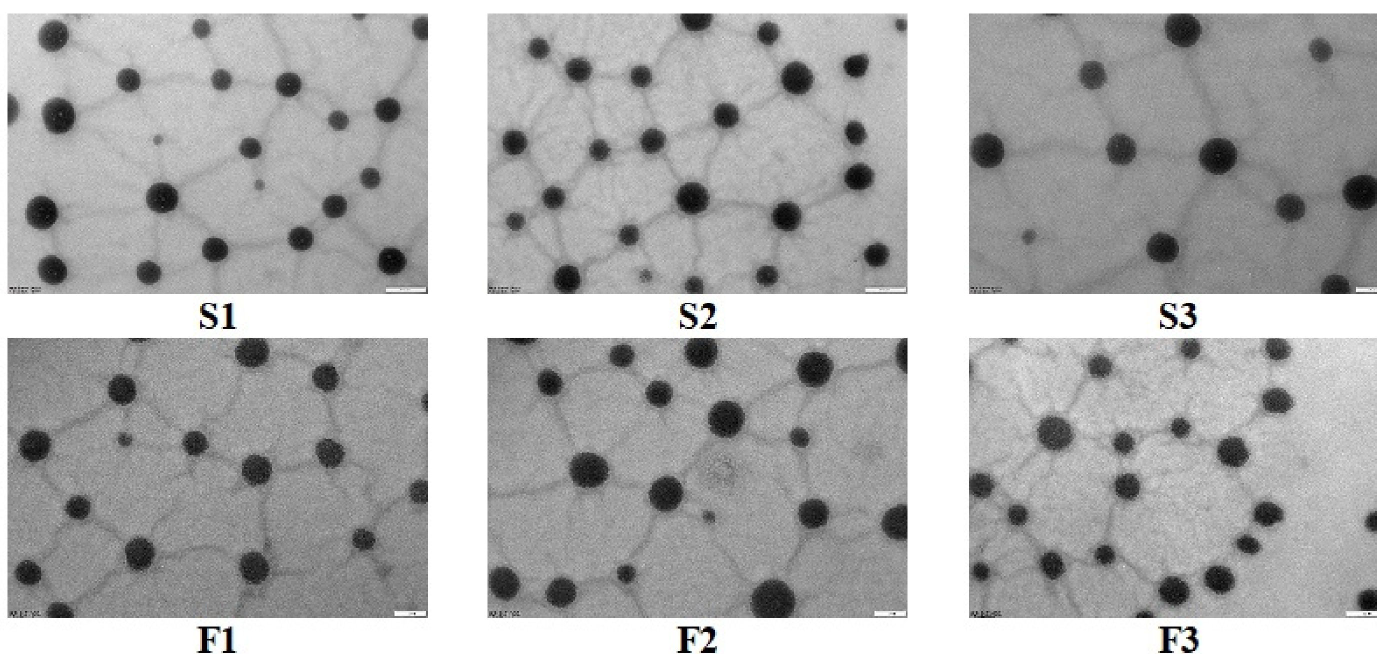

Figure 5: TEM images of SNEDDs and S-SNEDDS formulations.

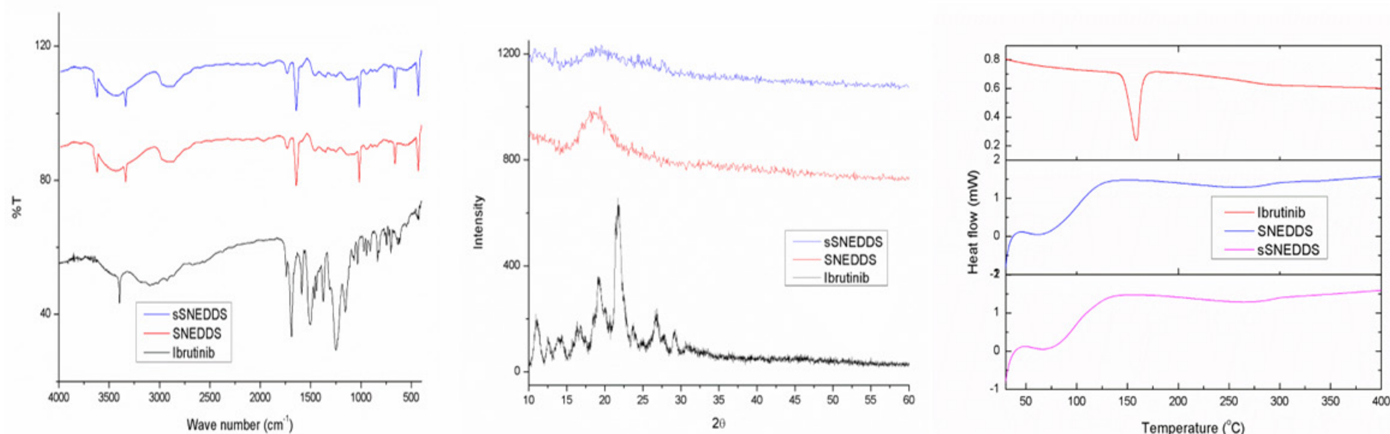

Figure 6: FTIR, XRD and DSC spectra of pure ibrutinib, SNEDDS and S-SNEDDs formulations.

Stability Studies

The thermodynamic stability of the SNEDDS formulation (S3) was assessed through temperature cycling, freeze-thaw cycles, and centrifugal stress testing. The formulation remained stable at both 4ºC and 40ºC, showing no evidence of phase separation or precipitation, thereby confirming its robustness during storage and transport. Under freeze-thaw cycles (-21ºC to +25ºC), it retained homogeneity and structural integrity, confirming resistance to extreme temperature fluctuations. Additionally, centrifugation at 3500 rpm showed no signs of instability, reinforcing its mechanical robustness. These findings establish SNEDDS (S3) as a stable and reliable formulation, suitable for long-term pharmaceutical applications.

The long-term stability of the optimized formulation was evaluated by storing it at refrigerated (4ºC) and room temperature (25ºC) conditions. No significant changes ($p<0.05$) in droplet size, zeta potential, or PDI were observed, indicating minimal degradation over time. The formulation maintained consistent physicochemical properties, ensuring stability, uniformity, and effectiveness throughout the study period (Table 4), confirming its suitability for long-term pharmaceutical applications.

Cytotoxicity Evaluation

The colorimetric MTT assay evaluates cell metabolic activity and was used to screen the test drug for apoptosis or necrosis in MCF-7 and PANC-1 cells. In an MTT safety test, the SNEDDS formulation with the safe ingredients exhibited nearly minimal cytotoxicity. As demonstrated in Figure 8, the pretreatment media containing the formulated product exhibited more than 97% cell viability, with cell death remaining under 3% across all concentrations. Figure 9 illustrates the morphological changes observed in MCF-7 and PANC-1 cancer cells following treatment with Ibrutinib pure drug (a & c) and the test formulation (b & d). Significant alterations in cell shape, detachment, and shrinkage were noted, indicating cytotoxic effects. The test formulation (b & d) exhibited a greater impact on cell morphology, suggesting enhanced therapeutic efficacy compared to the pure drug. Dose-response analysis was performed using a Four-Parameter Logistic (4PL) model in GraphPad Prism to estimate IC_{50} and Imax values. The pure ibrutinib exhibited marked cytotoxicity with IC_{50} values of approximately 18 µM (MCF-7) and 25 µM (PANC-1), while the S-SNEDDS formulation maintained over 97% cell viability across the tested concentrations, with the IC_{50} not reached within the experimental range, confirming a sustained-release and reduced burst exposure profile.

DISCUSSION

Solubility is a critical factor in SNEDDS formulation to prevent drug precipitation upon dilution with gastric fluids. Poorly soluble drugs with high solubility in SNEDDS may still precipitate when dispersed in aqueous media. Therefore, selecting an oil, surfactant, and co-surfactant with optimal solubilization capacity is essential. The results confirmed that Castor oil was the most effective oil for solubilizing ibrutinib, ensuring high drug loading capacity. Similarly, Kolliphor® RH 40 and PEG-600 exhibited strong solubilization and emulsification properties, making them ideal for the formulation. The oil phase plays a crucial role in the self-emulsification process, influencing droplet size and bioavailability. Medium- and long-chain triglycerides are favoured for their ability to improve drug absorption in the small intestine. Upon oral administration, triglycerides are broken down into monoglycerides and fatty acids, which stimulate endogenous biliary lipid release, further aiding drug solubilization. In this study, Castor oil was selected due to its high solubility for ibrutinib. The balance between solubilization capacity and emulsification efficiency was considered to ensure effective drug delivery. Surfactants are vital for lowering interfacial tension, facilitating the quick formation of emulsions. The ideal surfactant should have an HLB > 12 to form nanoemulsions with droplet sizes <200 nm. Kolliphor® RH 40 was found to be the best surfactant due to its excellent emulsification ability, high solubilization capacity, and stability in SNEDDS formulations. Similarly, co-surfactants enhance emulsification and interfacial

fluidity, reducing gastrointestinal irritation. Among the co-surfactants tested, PEG-600 provided the best emulsification efficiency, ensuring stable nanoemulsion formation.

The ternary phase diagram is an important tool for identifying the most efficient self-nanoemulsifying region, ensuring the formulation remains stable and readily disperses upon dilution. The results indicated that increasing the surfactant-to-co-surfactant ratio significantly facilitated nanoemulsion formation, likely by enhancing the decrease of interfacial tension and improving the stability of the droplets. The optimized formulation range ensures that ibrutinib remains in a dissolved state within the nanoemulsion, preventing precipitation upon dilution in gastric fluids. This systematic approach highlights the importance of using pseudo-ternary phase diagrams to fine-tune SNEDDS formulations, ultimately enhancing drug solubility, stability, and bioavailability.

Experimental design is a powerful approach for minimizing process variability and ensuring uniform particle size distribution and high formulation efficiency. Among various optimization techniques, the Box-Behnken design was chosen for its efficiency in evaluating nonlinear interactions between variables while reducing the number of experimental runs. The results showed that changes in the concentrations of Castor oil, Kolliphor® RH 40, and PEG-600 had a significant impact on both droplet size and encapsulation efficiency. Higher surfactant concentrations improved stability and emulsification, leading to smaller droplet sizes. Meanwhile, the oil phase played a crucial role in drug solubilization and nanoemulsion formation.

By systematically analyzing response surface plots and ANOVA results, an optimized SNEDDS formulation was identified, ensuring enhanced drug solubility, stability, and bioavailability. This approach highlights the importance of statistical modeling in pharmaceutical formulation development, enabling precise control over key formulation parameters for an effective drug delivery system. The mathematical equations derived from regression analysis illustrated how each factor influenced droplet size and encapsulation efficiency. Positive coefficients indicated synergistic effects, while negative coefficients suggested antagonistic interactions. Higher-order terms and interaction coefficients highlighted the complex relationships between variables. A backward elimination procedure confirmed the statistical significance ($p>0.01$) of both polynomial equations, as validated by ANOVA. These findings emphasize the importance of statistical modeling in optimizing SNEDDS formulation, providing a robust predictive framework for improving drug solubility, stability, and bioavailability.

The quadratic model demonstrated that Castor oil (A), Kolliphor® RH 40 (B), and PEG-600 (C) had a significant effect on droplet size, highlighting their importance in the SNEDDS formulation. The close correlation between predicted and observed values confirmed the model's reliability. Smaller droplet sizes correlate with faster drug release and enhanced absorption, making this optimized SNEDDS formulation highly effective. The insights gained from statistical modelling and response surface analysis provide a robust framework for optimizing droplet size, ensuring efficient drug delivery. The encapsulation efficiency of ibrutinib within SNEDDS is crucial for stability, bioavailability, and controlled drug release. The study found that surfactant concentration (B) had the greatest impact, likely due to its role in stabilizing the drug within the nanoemulsion.

The desirability function is a powerful statistical tool for simultaneously optimizing multiple response variables, ensuring an optimal balance between droplet size and encapsulation efficiency. The Box-Behnken design effectively modelled the relationships between formulation components, while response surface analysis provided insights into their interactions. This study demonstrates that the desirability function method, combined with experimental design tools, is an effective strategy for developing and refining SNEDDS formulations, ensuring enhanced drug solubility, stability, and bioavailability.

To determine the optimal HPMC K4M concentration, S-SNEDDS formulations were prepared with varying amounts of HPMC K4M (0.5%, 1.0%, 2.0%, and 5.0%). The results showed that higher HPMC concentrations improved precipitation inhibition, with no significant difference between 2.0% and 5.0%.

Table 3: Kinetic Analysis of Ibrutinib Release from SNEDDS and S-SNEDDS.

Model	Ibrutinib SNEDDS		Ibrutinib S-SNEDDS	
	R2	n	R2	n
Zero order	0.9477	0.2047	0.9468	0.2165
First order	0.8999	-0.0011	0.8942	-0.0012
Higuchi	0.8143	3.1158	0.8155	3.2982
Korsmeyer-Peppas	0.6437	25.22	0.6426	26.61

Table 4: Results of long-term stability study- Size distribution and zeta potential values.

Parameter	0 months		3 months		6 months	
	4±1ºC	25±2ºC	4±1ºC	25±2ºC	4±1ºC	25±2ºC
Droplet size (nm)	58.76±1.76	58.76±1.76	63.54±2.12	70.12±1.83	66.12±2.06	72.21±2.32
Zeta potential (mV)	-22.3±1.4	-22.3±1.4	-23.43±2.12	-23.21 ±1.54	-24.32±1.67	-23.82 ±1.74
PDI	0.097±0.005	0.097±0.005	0.123±0.005	0.112±0.005	0.132±0.005	0.138±0.005

$n=3$ ($p<0.05$).

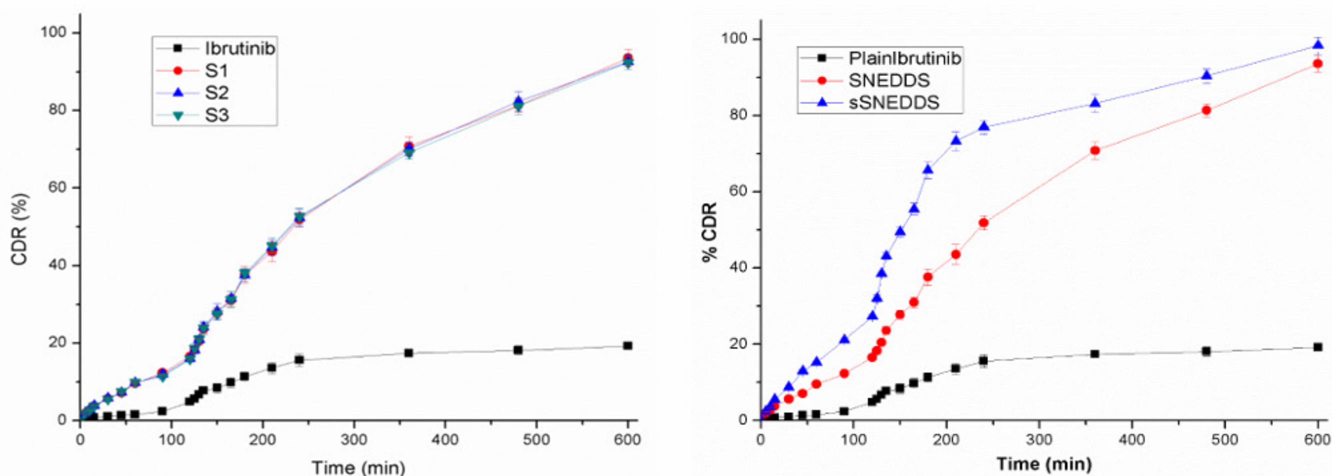

Figure 7: Diffusion profile of ibrutinib.

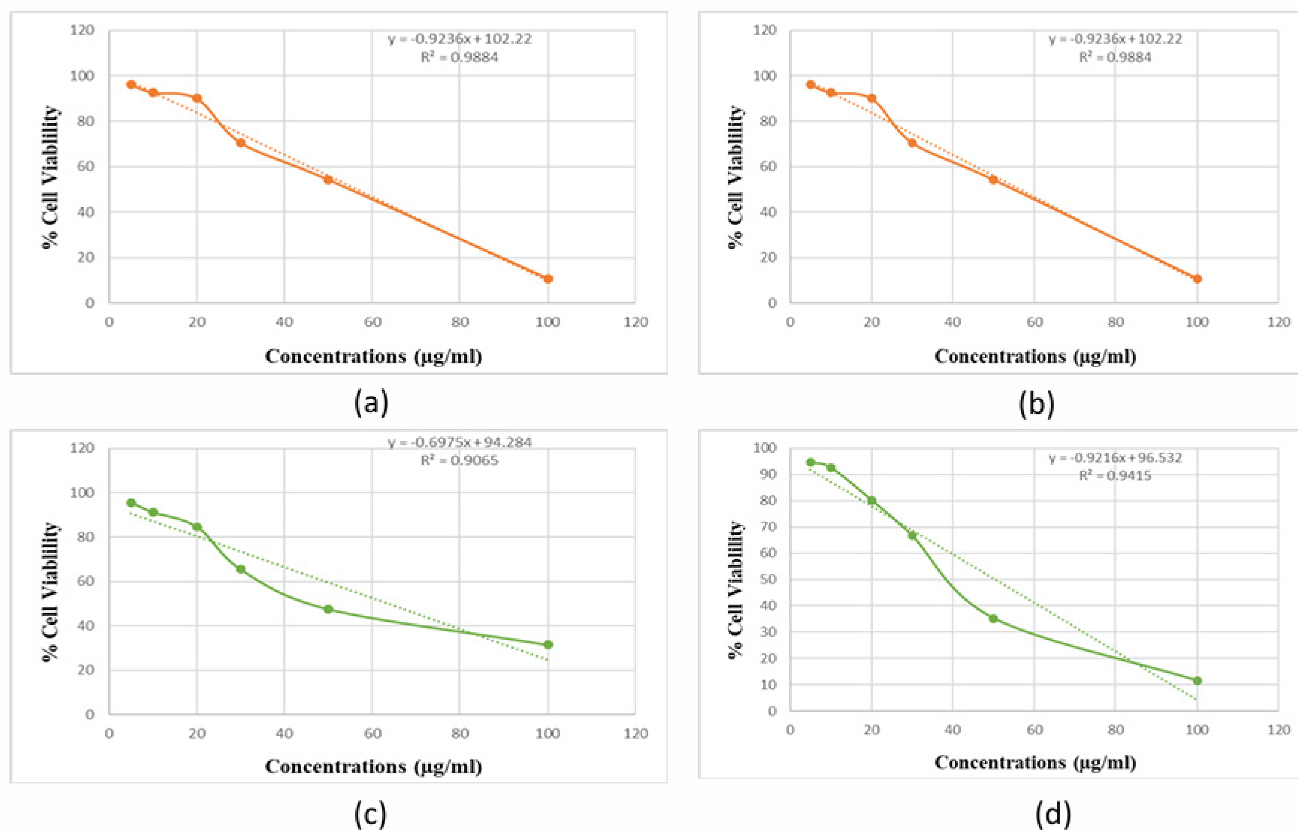

Figure 8: Cytotoxicity of Ibrutinib Pure drug (a & c) and test formulation (b & d) on PANC-1 and MCF-7 Cell line.

While the self-emulsification time increased slightly with higher HPMC content, all formulations still emulsified within 1 minute, indicating efficient self-emulsification. Given these results, 2% HPMC K4M was chosen as the ideal precipitation inhibitor for subsequent studies.

Smaller droplet sizes in S-SNEDDS indicate improved solubility and faster drug absorption. The negative zeta potential values indicate significant repulsive forces that help prevent aggregation, thereby improving long-term stability. TEM imaging confirmed the spherical morphology, ensuring uniformity in drug dispersion. FTIR analysis verified no chemical degradation, while XRPD and DSC confirmed the conversion of ibrutinib into an amorphous state, which is beneficial for enhanced solubility and bioavailability. The optimized SNEDDS and S-SNEDDS formulations successfully encapsulated ibrutinib, reducing droplet size, improving stability, and transforming the drug into an amorphous state, all of which contribute to enhanced solubility and absorption.

The self-nanoemulsifying nature of SNEDDS and S-SNEDDS facilitated rapid interface formation between the oil phase and dissolution medium, leading to faster drug release. The nano-sized globules increased the surface area, accelerating drug dissolution and absorption, while the amorphous transformation of ibrutinib reduced crystalline-related solubility limitations. Although SNEDDS significantly improved dissolution, S-SNEDDS demonstrated superior performance by effectively inhibiting precipitation and maintaining extended drug supersaturation, ensuring sustained high drug availability in gastrointestinal fluids. These findings demonstrate the effectiveness of S-SNEDDS in improving the oral bioavailability of ibrutinib, presenting it as a promising strategy for enhanced therapeutic outcomes.

This study investigated the dissolution behavior and release kinetics of ibrutinib from optimized SNEDDS and S-SNEDDS formulations. Both formulations exhibited similar release profiles, with regression coefficients approaching unity, particularly in zero-order kinetics, indicating a consistent dissolution rate over time. Mathematical modelling confirmed the zero-order release pattern, suggesting a steady and controlled drug release. Further analysis using Higuchi and Korsmeyer-Peppas plots revealed an n value indicative of super case II transport, pointing to a complex release mechanism involving both diffusion and matrix relaxation. These findings highlight the potential of SNEDDS and S-SNEDDS formulations to provide controlled, sustained release, enhancing the bioavailability and therapeutic efficacy of ibrutinib. While the *in vitro* release and kinetic modeling studies provide valuable insights into the drug release behavior of the optimized S-SNEDDS, the absence of *in vivo* pharmacokinetic data limits the translational relevance of these findings. Future work will focus on conducting detailed *in vivo* studies to establish pharmacokinetic-pharmacodynamic correlations and confirm the enhanced oral bioavailability and therapeutic efficacy of the formulation.

A stable SNEDDS formulation is essential for maintaining drug efficacy, uniformity, and shelf life. The absence of phase separation or precipitation under varied storage and mechanical stress conditions suggests strong emulsification properties and high formulation robustness.

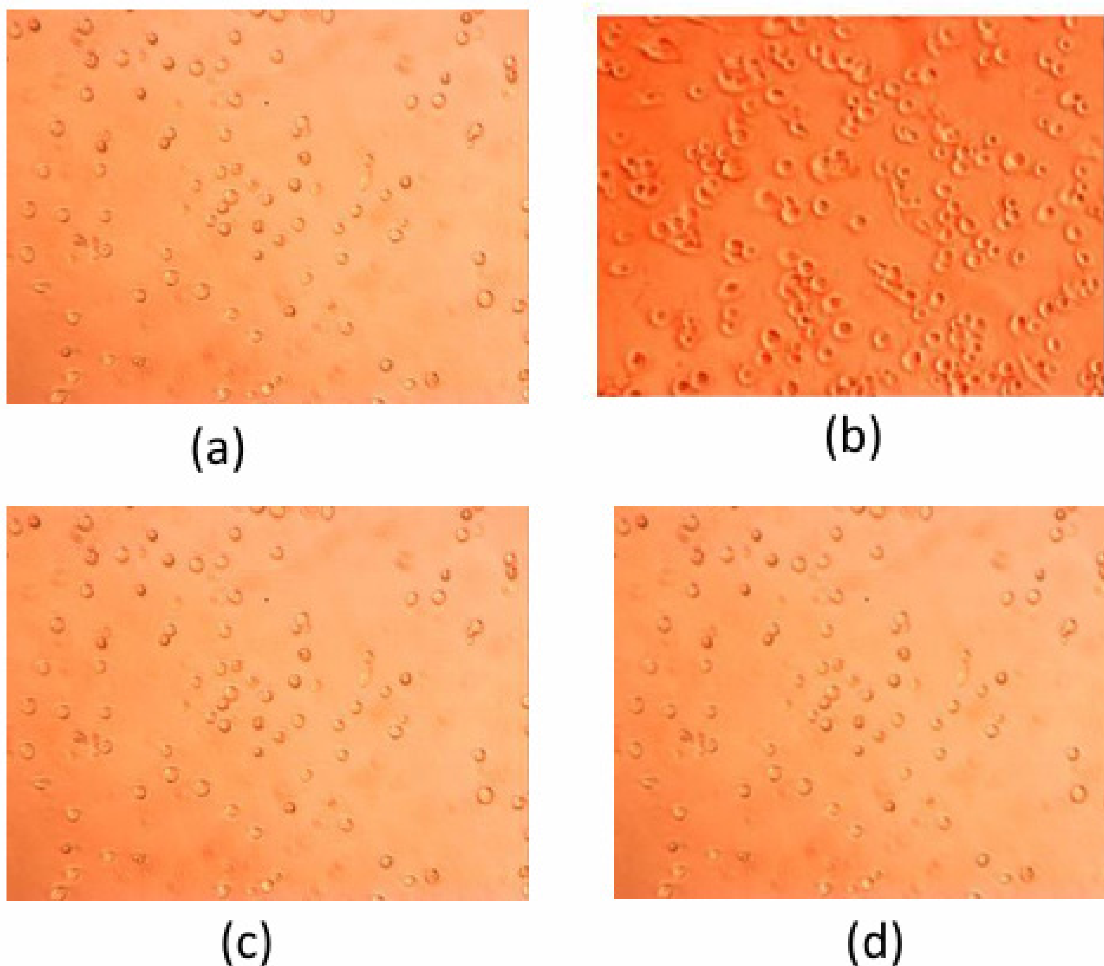

Figure 9: Morphological changes of MCF-7, PANC-1cancer cells when treated with Ibrutinib Pure drug (a & c) and test formulation (b & d).

The long-term stability study further confirmed that the optimized SNEDDS remains unaffected by environmental changes, ensuring reproducibility and effectiveness in drug delivery. These results reinforce the potential of SNEDDS (S3) as a reliable and stable carrier for ibrutinib, suitable for commercial pharmaceutical applications.

The colorimetric MTT assay serves as a standard technique for evaluating cell metabolic activity and identifying apoptosis or necrosis-inducing compounds. This method relies on NAD(P) H-dependent cellular oxidoreductase enzymes to convert yellow tetrazolium MTT into insoluble formazan crystals. The presence of these crystals serves as an indirect indicator of cell viability, as active, metabolically functional cells are capable of converting MTT into formazan. The amount of formazan formed correlates with the number of viable cells, offering a reliable method to assess cell health and metabolic activity.

In this study, the SNEDDS formulation exhibited minimal cytotoxicity, indicating its biocompatibility and safety. The pretreated media containing the formulated SNEDDS maintained over 97% cell viability, with less than 3% cell death across all concentrations, confirming the non-toxic nature of the formulation.

Figures 8 and 9 illustrate the cytotoxicity of pure Ibrutinib and the SNEDDS formulation on PANC-1 and MCF-7 cell lines, as well as the morphological changes observed post-treatment. These results indicate that the SNEDDS formulation significantly improves drug delivery while preserving cell viability, positioning it as a promising strategy for safe and effective cancer therapy.

CONCLUSION

This study successfully developed and optimized a supersaturable SNEDDS (S-SNEDDS) for ibrutinib, significantly enhancing its aqueous solubility and oral bioavailability. Using solubility screening, pseudo-ternary phase diagrams, and Box-Behnken design, an optimal formulation comprising castor oil, Kolliphor® RH 40, and PEG-600 was identified. The S-SNEDDS exhibited nano-sized droplets, high encapsulation efficiency, and excellent stability, while characterization studies confirmed the amorphous transformation of ibrutinib, contributing to improved dissolution. The formulation maintained sustained supersaturation, inhibited precipitation, and demonstrated minimal cytotoxicity, highlighting its biocompatibility. Overall, these findings establish S-SNEDDS as a promising strategy to enhance the oral delivery and therapeutic efficacy of ibrutinib, offering potential for improved cancer treatment outcomes.

ACKNOWLEDGEMENT

None.

ABBREVIATIONS

MDR: Multidrug resistance; **BTK:** Bruton's tyrosine kinase; **SNEDDS:** Self-nanoemulsifying drug delivery systems, **GI:** Gastrointestinal; **QbD:** Quality by Design; **S-SNEDDS:** Supersaturable self-nanoemulsifying drug delivery system; **RSM:** Response Surface Methodology; **BBD:** Box-Behnken Design; **PEGs:** Polyethylene glycols; **ANOVA:** Analysis of variance; **R^2:** Correlation coefficient; **3D:** Three dimensional; **2D:** Two dimensional; **HPMC K4M:** Hydroxypropyl methyl cellulose; **PVP K30:** Polyvinyl pyrollidine; **DLS:** Dynamic light scattering; **SGF:** Simulated gastric fluid; **TEM:** Transmission Electron Microscopy; **FTIR:** Fourier transformed infrared; **DSC:** Differential scanning calorimetry; **XRPD:** X-ray powder diffraction; **RI:** Refractive index; **SIF:** Simulated Intestinal Fluid; **PDI:** Polydispersity index; **ICH:** International Council for Harmonisation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The study focused on developing an optimized Supersaturable Self-Nanoemulsifying Drug Delivery System (S-SNEDDS) for ibrutinib, a poorly water-soluble drug used in cancer treatment, to enhance its oral bioavailability and anticancer efficacy. Optimization utilized a Box-Behnken Design (BBD), identifying Castor oil, Kolliphor® RH 40, and PEG-600 as optimal components.

The optimized S-SNEDDS demonstrated favorable characteristics, including a small droplet size (58.76 to 67.12 nm) and high encapsulation efficiency (61.56-87.22%). Characterization confirmed the amorphous nature of encapsulated ibrutinib, which aids dissolution, and the formulation exhibited rapid drug release following zero-order kinetics. Cytotoxicity evaluation showed the nanoformulation had lower cytotoxicity on MCF-7 and PANC-1 cells compared to the pure drug, suggesting enhanced therapeutic potential. These findings establish S-SNEDDS as a promising strategy for enhancing the oral delivery of ibrutinib.

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