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ORIGINAL ARTICLE

Formulation, optimization and characterization of self-emulsifying amorphous solid dispersion (SE-ASD) of Fusidic acid for enhanced antimicrobial efficacy

Akshay M. Guldagad¹, Dr. Jaydeep B. Pawar^{1*}

1. Department of Pharmaceutics, Shantiniketan College of Pharmacy, Dhotre (Bk), Ahilyanagar, Maharashtra, India- 414304.

Abstract

Objectives: Fusidic acid, a BCS Class II antibiotic, suffers from poor aqueous solubility and erratic oral bioavailability, limiting its clinical efficacy against MRSA and staphylococcal infections. This study aimed to develop, optimize, and characterize a self-emulsifying amorphous solid dispersion (SE-ASD) of Fusidic acid to enhance its dissolution and antimicrobial potential. **Methods:** Soybean oil, Tween 80, and Transcutol P were selected as oil, surfactant, and co-surfactant based on solubility screening and pseudo-ternary phase diagram construction. A three-factor, three-level Box-Behnken design was employed to optimize seventeen SNEDDS formulations, with droplet size and cumulative drug release at 30 minutes as dependent variables. The optimized SNEDDS was solidified using PVP K30 via solvent evaporation to yield SE-ASD, which was characterized by DSC, FTIR, PXRD, reconstitution studies, in vitro dissolution, and accelerated stability testing. **Results:** The quadratic model demonstrated excellent predictability ($R^2 = 0.9997$ for droplet size; $R^2 = 0.9822$ for drug release). Formulation F16 was selected as optimal based on maximum desirability (1.000), yielding droplet size of 101.3 ± 1.5 nm, percentage transmittance of $99.1 \pm 0.2\%$, and drug release of $96.10 \pm 0.74\%$ at 30 minutes. SE-ASD confirmed complete amorphous conversion by PXRD and achieved $96.84 \pm 0.81\%$ drug release at 30 minutes. Accelerated stability studies confirmed physicochemical stability over three months at $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$. **Conclusion:** The developed SE-ASD successfully enhanced dissolution of fusidic acid through synergistic amorphization and self-emulsification, demonstrating significant potential for improved oral bioavailability and clinical translation in staphylococcal infection management.

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***Corresponding author: Dr. Jaydeep B. Pawar**, Department of Pharmaceutics, Shantiniketan College of Pharmacy, Dhotre (Bk), Ahilyanagar, Maharashtra, India- 414304, Email: jppharmaresearch@gmail.com

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INTRODUCTION

Staphylococcus aureus and other gram-positive bacteria are a major public health problem in the 21st century. Together, the SSTIs, osteomyelitis, bacteremia and device-associated infections account for millions of hospitalizations each year and are a significant burden on health systems globally [1]. The rise and widespread appearance of methicillin-resistant *Staphylococcus aureus* (MRSA) have severely restricted the therapeutic options available with the emergence of several classes of antibiotic drugs that are clinically ineffective [2]. By 2050, antimicrobial resistance is estimated to kill about 10 million people each year, and cost the global economy more than \$100 trillion dollars [3]. Limited access to parenteral antibiotic treatment also intensifies morbidity and mortality due to infectious diseases in low- and middle-income countries [4]. Although several types of antibiotics are available, subtherapeutic levels of these drugs at the site of infection remain a clinical challenge and an accepted fact of life for poor bioavailability in the oral cavity [5]. Oral formulations that are conventional usually do not maintain sufficient systemic exposure, which is directly linked to treatment failures and the development of resistance [6]. These complementary constraints call for novel formulation approaches to maximize the delivery of oral drug candidates [7].

Fusidic acid is a naturally occurring steroidal antibiotic found in *Fusidium coccineum* and is considered to have a high level of both bacteriostatic and bactericidal activity against gram-

positive organisms, including *S. aureus*, MRSA, and coagulase-negative staphylococci. Fusidic acid has a tetracyclic steroid backbone that is highly lipophilic with a log P of around 5.4 and a very low aqueous solubility of less than 0.1mg/mL at physiological pH and is considered a BCS Class II drug [8,9]. The mechanism of action is unique, involving selective inhibition of bacterial protein synthesis via stabilization of the elongation factor G (EF-G)–ribosome–GDP complex leading to the arrest of bacterial proliferation without interfering with eukaryotic protein synthesis [10]. The clinical use of oral fusidic acid is significant for the treatment of staphylococcal osteomyelitis, wound infection and MRSA-related infections especially in combination therapy, but is greatly limited by its erratic gastrointestinal absorption, large extent of first-pass metabolism, enterohepatic recycling and highly variable bioavailability [11]. In recent pharmacokinetic studies, deficiencies of the available conventional tablet formulations have been confirmed in order to be able to achieve a therapeutic plasma level consistently [12].

Self-emulsifying amorphous solid dispersion (SE-ASD) technology is a scientifically sound and clinically promising drug delivery platform to overcome these drawbacks [13]. SE-ASD is a synergic approach of two well-validated solubility-enhancement strategies: amorphous solid dispersion (ASD) and self-emulsifying drug delivery systems (SEDDS) [14]. In an ASD, the drug molecules are molecularly surrounded by a hydrophilic polymeric carrier in PDS; the apparent solubility in the

mixture is greatly increased and the IDR is significantly higher than the crystalline form [15]. Oil-in-water nanoemulsion is a spontaneous self-emulsification process, with simultaneous incorporation of lipidic excipients (oils, surfactant(s), co-surfactant(s)) into the solid matrix that increases the permeability of the membranes, the uptake in the lymphatic system and the oral absorption [16]. This dual mechanism is successful in overcoming the interrelated challenges of low aqueous solubility and low gastrointestinal permeability that are typical for BCS Class II compounds like fusidic acid. The recent use of these novel manufacturing methods such as hot-melt extrusion, spray drying and solvent evaporation has enabled the scalable and reproducible production of a novel SE-ASDs that offer considerably improved solid state stability and in vivo performance characteristics, while also alleviating practical formulation and patient compliance issues [17].

The present work is focused to formulate, optimize and characterize an SE-ASD of Fusidic acid with the main objective of improving its oral bioavailability and antimicrobial efficacy. Importantly, the study aims to identify optimal polymer-lipid-surfactant combinations, to characterize the solid-state properties, to evaluate in vitro dissolution characterization and self-emulsification performance, and to investigate antimicrobial activity against clinically relevant staphylococcal strains to address an important gap in the rational formulation of poorly water-soluble antibiotics.

MATERIALS AND METHODS

MATERIALS

The pharmaceutical grade purity $\geq 98\%$ of fusidic acid was purchased from Sigma-Aldrich (St. Louis, USA). Soybean oil (NF grade) was purchased from Spectrum Chemical (Gardena, USA). Tween 80 (polysorbate 80, HLB value 15.0) and Transcutol P (diethylene glycol monoethyl ether, purity $\geq 99\%$) were kindly gifted by Gattefossé (Saint-Priest, France). PVP K30 (PVP, molecular weight = 40,000 Da) was purchased from BASF SE (Ludwigshafen, Germany). Potassium dihydrogen phosphate and disodium hydrogen phosphate (analytical grade) for the preparation of phosphate buffer was purchased from Merck Life Sciences Pvt. Ltd. Mumbai, India. The sodium lauryl sulfate (analytical grade) was purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Potassium bromide (FTIR grade, purity $\geq 99\%$) was purchased from Sigma Aldrich (St. Louis, USA). Methanol, HPLC grade, was bought from Merck Life Sciences Pvt. Ltd. (Mumbai, India). Hard gelatin capsules (size 0) were purchased from Capsugel (Greenwood, USA). All other chemicals and reagents were used as analytical grade and procured from commercial sources.

METHODS

Calibration Curve Determination of Fusidic Acid

Fusidic acid stock solution (1000 $\mu\text{g/mL}$) was prepared by weighing out 10 mg of the drug with an accuracy of 0.1 mg and dissolving it in HPLC grade methanol in a 10 mL volumetric flask. The stock

solution was diluted with methanol to make the standard solutions of 2, 4, 6, 8, 10, and 12 µg/mL. The absorbance of each solution was taken in triplicate (n=3) in a UV-visible spectrophotometer (Systronic UV-2201, Systronic Instruments Pvt. Ltd., Ahmedabad, India) using methanol as blank at the λ_{max} determined. A calibration curve was formed by taking absorbance versus concentration and linear regression analysis ($y = mx + c$) was used to check linearity. An acceptable correlation coefficient (r^2) was ≥ 0.999 . Data are presented as mean $\pm$ standard deviation (SD) [18,19].

Solubility Study

The solubility of fusidic acid was measured in a number of oils, surfactants, co-surfactants and water by the shake flask method. 5 mL of each vehicle was placed in screw-capped glass vials, an excess amount of fusidic acid (~50–100 mg) was added to each, vortexed for 5 minutes, and equilibrated in a thermostatic water bath shaker for 72 hours at $37 \pm 0.5^\circ\text{C}$ and 100 rpm. Samples were centrifuged for 15 minutes at 5000 rpm, then the supernatant was filtered through 0.45 µm membrane filter and a proper amount of methanol was added, and measured by UV-visible spectrophotometry with a predetermined λ_{max} with the established calibration curve. The results of solubility values were presented as mean $\pm$ SD (n=3) in mg/mL [20].

Construction of Pseudo-Ternary Phase Diagram

According to solubility data, Tween 80 was chosen as the surfactant, Transcutol P as the co-surfactant and the oil phase was soybean oil. Surfactant/co-surfactant (Smix) ratios of 1:1, 1:2, 2:1 and 3:1

(w/w) were tested. Different proportions of oil-to-Smix (1:9 to 9:1, w/w) were tested for each Smix ratio. Each mixture (100 mg) was dispersed in 100 mL distilled water ($37 \pm 0.5^\circ\text{C}$) with gentle magnetic stirring at 100 rpm and the emulsification behavior was evaluated visually for clarity, appearance, and emulsification time. Mixtures that formed clear or translucent emulsions in less than 2 minutes were considered to be self-emulsifying. Pseudo-ternary phase diagrams were created by the software Ternaryplot.com to determine the optimum self-emulsifying region [21].

Differential Scanning Calorimetry

DSC was used for thermal behavior and compatibility of drugs with excipients. Selected excipients were gently triturated with pure fusidic acid for 10 minutes to give the physical mixture and fusidic acid. The samples (3 to 5 mg) were placed in aluminum pans and heated under nitrogen purge (50 mL/min) at a rate of $10^\circ\text{C}/\text{min}$ and analyzed between 30 and 300°C with DSC instrument calibrated with indium standard. Thermograms were examined for endotherms, glass transition temperature, and any change or loss of characteristic peaks that may be indicative of drug-excipient interactions [22].

Fourier Transform Infrared Spectroscopy

FTIR spectroscopy was used to determine the chemical compatibility between the fusidic acid and excipients. Trituration for 10 minutes was used for the preparation of pure drug and physical mixture. 2–3 mg of each sample were mixed with 100 mg of dried KBr and were pressed in a hydraulic press

under 10 tons pressure for 2–3 minutes to form KBr pellets. Spectra were collected from 4000–400 cm^{-1} at a resolution of 4 cm^{-1} and with 32 scans per sample. The characteristic peaks –OH, C=O, –COOH and aliphatic C–H stretching were analyzed and any shifting, broadening or disappearance of the peaks in physical mixture was noted when compared to pure drug to confirm compatibility [23].

Experimental Design

The optimization of the formulation of SE-ASD was done using Design-Expert software (Version 13, Stat-Ease Inc., Minneapolis, USA) by employing Box-Behnken Design (BBD). Three independent variables namely oil concentration (X_1 : Soybean oil, % v/v), surfactant concentration (X_2 : Tween 80, % v/v) and co-surfactant concentration (X_3 : Transcutol P, % v/v) were chosen at three levels coded as –1, 0 and +1, respectively, in the self-emulsifying region of Smix ratio 3:1. A total of 17 experimental runs (with 5 center points) were produced from the design. Drug release at 30 minutes (Y_2) and droplet size (Y_1) were chosen as the dependent variables. The polynomial equation that was used to establish the relationship between variables was:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2$$

ANOVA, regression analysis and three dimensional response surface plots were used to determine the optimum formulation composition [24].

Table 1: Box-Behnken Design - Independent and Dependent Variables with Levels and Goals

Dependent Variable	Levels		
	Level -1 (Low)	Level 0 (Medium)	Level +1 (High)
Oil (Soybean oil) (%v/v)	6	34	62
Surfactant (Tween 80) (%v/v)	21	28	35
Co-surfactant (Transcutol P) (%v/v)	7	9	11
Dependent Variables			Goals
Droplet size			Minimize
Cumulative drug release at 30 min			Maximize

Formulation of Self-Nanoemulsifying Drug Delivery System

The Box-Behnken design was used to prepare 17 SNEDDS formulations of fusidic acid. Fusidic acid (250 mg) was dissolved in the calculated amount of soybean oil by gently heating at 40-50°C with magnetic stirring until it was completely dissolved. The quantity of Tween 80 and Transcutol P added was varied according to each experimental run, and the mixture was then vortexed for 10–15 minutes at room temperature to obtain complete mixing. The liquid SNEDDS formulations were subsequently left in the amber coloured glass vials and kept at room temperature away from light for further evaluations.

Table 2: Formulation Composition of SNEDDS Batches as per Box-Behnken Design

Batch	Factor 1: Oil % v/v	Factor 2: Surfactant % v/v	Factor 3: Co-surfactant % v/v	Fusidic Acid (mg)
F1	34	35	7	250
F2	34	28	9	250
F3	6	28	7	250

F4	62	28	7	250
F5	6	35	9	250
F6	34	35	11	250
F7	34	28	9	250
F8	62	28	11	250
F9	6	21	9	250
F10	34	21	7	250
F11	62	21	9	250
F12	34	28	9	250
F13	34	28	9	250
F14	34	21	11	250
F15	34	28	9	250
F16	6	28	11	250
F17	62	35	9	250

Solubility Study of Fusidic Acid in Different Polymers

The solubility of fusidic acid in various polymers PVP K30, PVP K90, HPMC E5, HPMC E15, Soluplus, PVP-VA 64, Eudragit EPO, and Poloxamer 407 — was determined by the solvent evaporation method. Each polymer (10, 20, 30, 40, 50 and 100 mg) was mixed with fusidic acid (10 mg) in separate glass vials to prepare drug:polymer ratios of 1:1, 1:2, 1:3, 1:4, 1:5 and 1:10 respectively. Methanol (5 mL) added, and mixed at room temperature for 30 minutes until dissolved. The solvent was evaporated in a hot air oven at 40°C and dried films were kept at room temperature for 24 hours. Visual and polarized light microscopy (40×) were used to examine the samples for drug crystallization. The minimum concentration at which complete amorphous conversion was attained was noted and the polymer with maximum solubilizing ability at minimum concentration was chosen for SE-ASD preparation [25].

Preparation of Self-Emulsifying Amorphous Solid Dispersion

The optimized SNEDDS composition was used to prepare SE-ASD of Fusidic acid by solvent evaporation technique. Solubilized fusidic acid in an optimized liquid SNEDDS consisting of 50 mg of fusidic acid, soybean oil, Tween 80 and Transcutol P was vortexed for 10 minutes. The liquid SNEDDS was mixed with PVP K30 solution (15% w/v) in absolute ethanol under magnetic stirring and the resulting mixture was stirred under constant stirring at 500 rpm for 60 minutes with a SNEDDS:polymer ratio (w/w) of 1:3. The evaporation of solvents was carried out under reduced pressure after 45°C in a rotary evaporator, leaving the lipidic components inside the polymer matrix. The resulting semi-solid material was dried again in a vacuum oven at 40°C for 2 hours, crushed with a mortar and pestle, passed through a #60 mesh sieve and kept in airtight amber colored containers with desiccant at room temperature until further testing [26].

Visual Assessment and Clarity

The 100 mg SNEDDS formulation was diluted with 100mL distilled water ($37 \pm 0.5^{\circ}\text{C}$), and was observed for ease of emulsification, appearance, phase separation and emulsion stability under gentle magnetic stirring at 100 rpm. Self nanotechnology systems that were able to form a clear/translucent emulsion within 2 min and did not exhibit any instability were taken as a successful system [27,28].

Emulsification Time

SNEDDS formulation (100 mg) was dropped into 100 mL distilled water at 100 rpm and $37 \pm 0.5^\circ\text{C}$ and the time required to reach complete emulsification recorded with the help of the digital stopwatch. Three measurements were taken ($n=3$) and formulations with emulsification time < 2 min were regarded as efficient [29].

Percentage Transmittance

All the SNEDDS formulation (100 mg) was dissolved in 100 mL of distilled water ($37 \pm 0.5^\circ\text{C}$) and then equilibrated for 5 minutes. The percentage transmittance was measured at 650 nm in a quartz cuvette (1 cm path length) with distilled water as blank in a UV-visible spectrophotometer (Systronic UV-2201, Ahmedabad, India). The values of $>90\%$ were transparent nanoemulsions, 80–90% translucent and $<80\%$ coarse emulsions. The results are presented as mean $\pm$ SD ($n=3$) [30].

Droplet Size and Zeta Potential

Dynamic light scattering and electrophoretic light scattering were performed to determine droplet size and zeta potential, respectively, of the SNEDDS formulations prepared using Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Formulations (100 mg) were made into 100 mL solution in distilled water ($37 \pm 0.5^\circ\text{C}$) and equilibrated for 5 minutes. The droplet size was determined at 25°C at 90° scattering angle and presented as Z-average diameter (nm). A folded capillary cell (DTS1070) was used to measure the zeta potential at 25°C in millivolts (mV). Droplet size <200 nm and zeta potential $> \pm 30$ mV were considered as stable

formulation. All measurements were done in triplicates ($n=3$) and presented as mean $\pm$ SD [31].

pH Measurement

All SNEDDS formulations (100 mg) were diluted with distilled water ($37 \pm 0.5^\circ\text{C}$) and equilibrated for 5 minutes. The pH was determined by a calibrated digital pH meter (Equip-Tronics, India) with standard buffer solutions (pH 4.0, 7.0 and 9.2). Each experiment was repeated three times ($n=3$) and the data are presented as means $\pm$ SD [32].

Drug Content

The formulation equivalent to 10 mg of fusidic acid was dissolved in methanol by sonication for 15 min in a 10 mL volumetric flask and filtered through the $0.45\ \mu\text{m}$ membrane filter before absorbance was measured at λ_{max} on a UV-visible spectrophotometer using methanol as blank. The drug contents were determined by using a calibration curve and reported as percent of theoretical. The formulations in the ranges of 95–105% were deemed to be acceptable. Data are presented as mean $\pm$ SD ($n=3$) [33].

Thermodynamic Stability Studies

Thermodynamic stability was evaluated by three consecutive stress tests: heating-cooling cycle (6 cycles from 4°C to 45°C , 48 h each cycle); centrifugation test (3500 rpm, 100-fold dilution, 30 min each cycle); and freeze-thaw cycle (3 cycles between -21°C and $+25^\circ\text{C}$, 24 h each cycle). The formulations were tested for phase separation, turbidity, creaming and precipitation of the drugs. Formulations that met all three tests were chosen for additional testing [34].

***In-vitro* Dissolution Study**

The *in-vitro* drug release from the optimized SNEDDS was tested in the USP Type II dissolution apparatus (paddle method) by dissolving in 900 mL phosphate buffer pH 6.8 with 0.5% (w/v) sodium lauryl sulfate to ensure sink conditions at $37 \pm 0.5^\circ\text{C}$ at 50 rpm. Test was SNEDDS equivalent to 50 mg of fusidic acid in hard gelatin capsules (size 0) while control was pure fusidic acid (50 mg) in hard gelatin capsules (size 0). Aliquots (5 mL) were removed at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes, filtered through a $0.45\ \mu\text{m}$ membrane filter and spectrophotometrically analyzed at λ_{max} , with the same volume of fresh pre-warmed medium being added. The calibration curve was used to calculate cumulative percentage drug release and plotted against the time. All studies were repeated three times ($n=3$) and presented as mean $\pm$ SD [35].

Drug Content

SE-ASD powder equivalent to 10mg of fusidic acid was dissolved in methanol by sonication for 20 minutes in a 10 mL volumetric flask, filtered through $0.45\ \mu\text{m}$ membrane filter and absorbance was measured at λ_{max} on UV-visible spectrophotometer, blanked with methanol. The percentage of theoretical drug content was determined from the calibration curve and expressed as the content of the drug. The percent that fell within 95-105% of the target was deemed to be acceptable. Data is presented as mean $\pm$ SD ($n=3$) [36].

Powder X-Ray Diffraction

PXRD was used to determine that the SE-ASD contained either amorphous or crystalline fusidic acid. The X-ray diffraction pattern of pure fusidic acid, physical mixture, PVP K30, freeze-dried SNEDDS and SE-ASD powder were obtained using X-ray diffractometer (Bruker D8 Advance, Germany) at Cu K α radiation (40 kV and 40 mA). The samples were scanned at ambient temperature between 5° – 50° with $2^\circ/\text{min}$ scan rate and 0.02° step size. Significant disappearance or reduction of the characteristic peaks associated with the crystallinity of fusidic acid was established on the SE-ASD [37].

Reconstitution Study

To evaluate the solidification effect on the self-emulsifying properties, 100 mg of the original SNEDDS was dispersed in 100 mL of distilled water (37 ± 0.5) to form the SE-ASD powder. The emulsification time, appearance and phase separation were visually monitored. The percentage transmittance value was measured at 650 nm using distilled water as blank and the droplet size was measured by dynamic light scattering. Comparisons were made between the results obtained and the original liquid SNEDDS. Studies were conducted three times ($n=3$) and mean $\pm$ SD was reported [38].

***In-vitro* Dissolution Study**

The evaluation of drug release from SE-ASD was made using USP Type II dissolution apparatus (paddle method) in which phosphate buffer pH 6.8 with 0.5% (w/v) sodium lauryl sulfate was used as dissolution media at $37 \pm 0.5^\circ\text{C}$ at 50 rpm. SE-ASD

powder equivalent to 50 mg of hard gelatin capsule size 0 of fusidic acid was tested along with its optimized liquid SNEDDS as well as physical mixture and pure fusidic acid as controls. Aliquots (5 mL) were drawn at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes after passing through a 0.45 μ m membrane filter, substituted with an equal volume of fresh pre-warmed medium and measured spectrophotometrically at λ_{max} . Cumulative percentage drug release was determined and plotted against time to perform kinetic modelling for the determination of drug release mechanism. All studies were repeated three times (n=3) and the results reported as mean $\pm$ SD [39].

Accelerated Stability Studies

The accelerated stability testing was carried out in accordance with guidelines in ICH Q1A(R2) at $40 \pm 2^\circ\text{C}$ at $75 \pm 5\%\text{RH}$ for 3 months in a stability chamber. SE-ASD powder was stored in sealed, amber glass containers that included desiccant. The samples collected at 0, 1, 2 and 3 months were assessed regarding their physical appearance, drug content, reconstitution efficiency, percentage transmittance, droplet size, in vitro drug release and solid state characterization by PXRD and DSC to check for any recrystallization. Comparisons of data with the initial values were made, and statistical significance was evaluated using one-way ANOVA [40].

Statistical Analysis

The data from experiments are reported as mean $\pm$ SD (standard deviation). The Box-Behnken design data were subjected to analysis by Design-Expert

software (Version 13, Stat-Ease Inc., Minneapolis, USA). One-way ANOVA followed by Tukey's post hoc test was used to determine the statistical differences between formulations ($p < 0.05$). All experiments were repeated three times (n=3) [41,42].

RESULTS AND DISCUSSION

RESULTS

Calibration Curve Determination

The calibration curve of fusidic acid in methanol was created between the concentrations 2 - 12 $\mu\text{g/mL}$ and is shown in Figure 1. The curve was found to be linear between absorbance and concentration with the regression equation $y = 0.0198x + 0.0015$ and correlation coefficient ($R^2 = 0.998$) showed that the method is well suited for quantitative analysis of the samples used in the study.

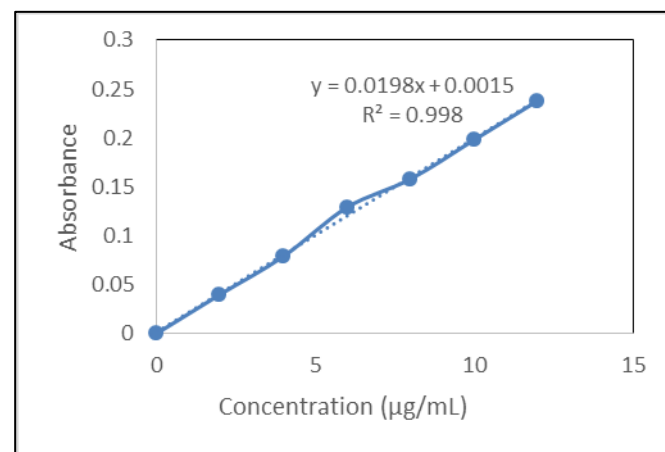


Figure 1: Calibration Curve of Fusidic acid in methanol

Solubility Study

Solubilities of fusidic acid in different vehicles are given in Table 3. Soybean oil had the highest solubility ($15.70 \pm 0.42 \text{ mg/mL}$) while Transcutol P

(269.20 $\pm$ 5.32 mg/mL) and Tween 80 (135.30 $\pm$ 2.45 mg/mL) demonstrated maximum solubility among the co-surfactants and surfactants, respectively. The solubility of fusidic acid in distilled water was 0.08 $\pm$ 0.01 mg/mL, which indicates that it is very poorly soluble and therefore belongs to the BCS Class II. Therefore, soybean oil, Tween 80 and Transcutol P were chosen for the preparation of SNEDDS.

Table 3: Solubility Study of Fusidic Acid in Different Solvents

Sr. No.	Solvent	Solubility (mg/mL)	Inference
Natural Oils and Lipids			
1	Sesame oil	6.70 $\pm$ 0.24	Sparingly soluble
2	Olive oil	10.26 $\pm$ 0.11	Sparingly soluble
3	Castor oil	10.30 $\pm$ 0.18	Sparingly soluble
4	Soybean oil	15.70 $\pm$ 0.42	Sparingly soluble
5	Groundnut oil	4.60 $\pm$ 0.15	Slightly soluble
6	Linseed oil	12.60 $\pm$ 0.33	Sparingly soluble
7	Corn oil	6.09 $\pm$ 0.24	Sparingly soluble
8	Sunflower oil	8.45 $\pm$ 0.28	Sparingly soluble
Surfactants			
9	Tween 80	135.30 $\pm$ 2.45	Freely soluble
10	Tween 20	69.40 $\pm$ 1.82	Soluble
11	Span 80	17.15 $\pm$ 0.34	Sparingly soluble
12	Span 20	84.90 $\pm$ 1.95	Soluble
13	Labrasol	86.70 $\pm$ 2.12	Soluble
14	Cremophor EL	93.50 $\pm$ 2.38	Soluble
15	Labrafil	96.70 $\pm$ 2.54	Soluble
16	Kolliphor RH40	78.25 $\pm$ 1.87	Soluble
Co-surfactants			
17	Ethanol	215.40 $\pm$ 4.56	Freely soluble
18	Transcutol P	269.20 $\pm$ 5.32	Freely soluble
19	Propylene glycol	125.80 $\pm$ 3.24	Freely soluble
20	Glycerol	1.94 $\pm$ 0.14	Slightly

			soluble
21	PEG 400	142.60 $\pm$ 3.78	Freely soluble
22	Isopropyl alcohol	198.30 $\pm$ 4.12	Freely soluble
Aqueous Solvents			
23	Distilled water	0.08 $\pm$ 0.01	Practically insoluble
24	Phosphate buffer pH 6.8	0.72 $\pm$ 0.05	Very slightly soluble
25	Phosphate buffer pH 7.4	0.84 $\pm$ 0.06	Very slightly soluble
26	0.1 N HCl	0.15 $\pm$ 0.02	Practically insoluble
27	Simulated gastric fluid pH 1.2	0.18 $\pm$ 0.03	Practically insoluble
28	Simulated intestinal fluid pH 6.8	0.65 $\pm$ 0.04	Very slightly soluble

Construction of Pseudo Ternary plot generation

The pseudo-ternary phase diagrams were obtained at four Smix ratios are shown in Fig. 2. The self-emulsifying zones (shaded areas) were different for all the ratios and were the widest in Smix 3:1 (D) suggesting that the emulsification ability was the highest when the proportion of the surfactant was the highest. Smix 2:1 (C) had a similarly large self-emulsifying area; ratios of 1:1 (A) and 1:2 (B) had relatively narrow self-emulsifying zones. Therefore, Smix 3:1 was chosen to be further developed into a formulation.

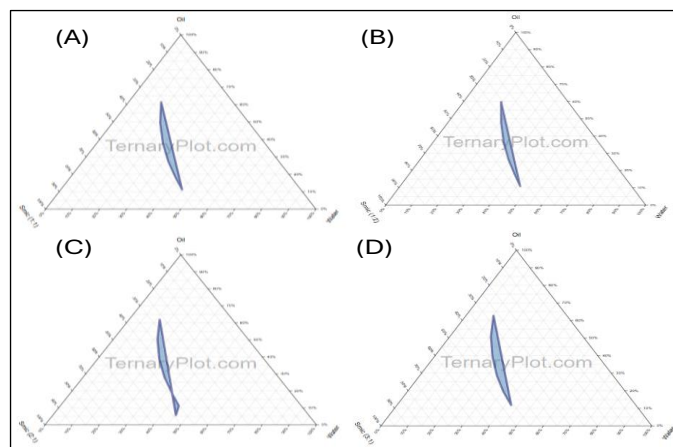


Figure 2: Pseudo-ternary phase diagrams of fusidic acid SNEDDS at surfactant-to-co-surfactant (Smix) ratios of (A) 1:1, (B) 1:2, (C) 2:1, and (D) 3:1. Shaded regions represent the self-emulsifying zone.

DSC Analysis

Figure 3 shows DSC thermograms of pure and physical mixtures of fusidic acid. The sharp endothermic peak at 192.54°C was observed in pure fusidic acid (A), indicating that it was crystalline. In the physical mixture (B) the characteristic fusidic acid melting peak was not seen to change significantly, which was attributed to the absence of drug-excipient incompatibility.

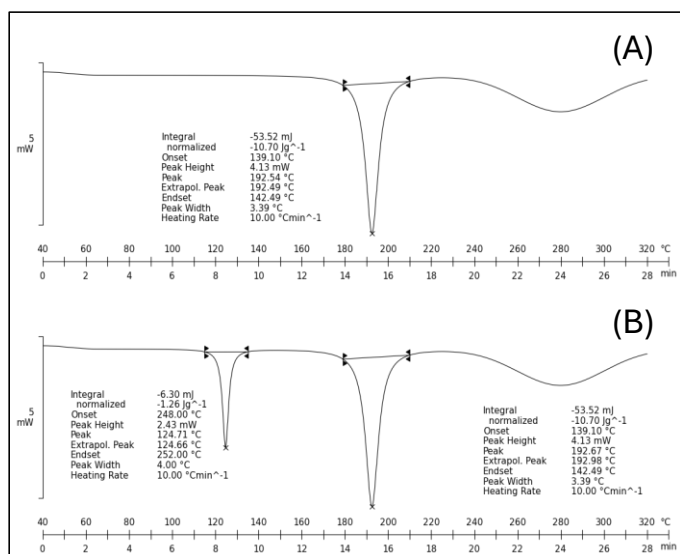


Figure 3: DSC Spectra of (A) Pure Drug [192.54] and (B) Physical Mixture [124.71 and 192.67]

FTIR Analysis

The FTIR of pure fusidic acid and physical mixture of fusidic acid with excipients are shown in Figure 4. Pure fusidic acid (A) displayed characteristic peaks at 3476.63 cm^{-1} (–OH stretching), 2951.70 and 2872.39 cm^{-1} (aliphatic C–H stretching), and 1798.96 cm^{-1} (C=O stretching). For the physical mixture (B), no significant shifts or disappearance of main peaks was observed and all the peaks were maintained at the same position indicating the

physicochemical compatibility of the selected excipients and fusidic acid.

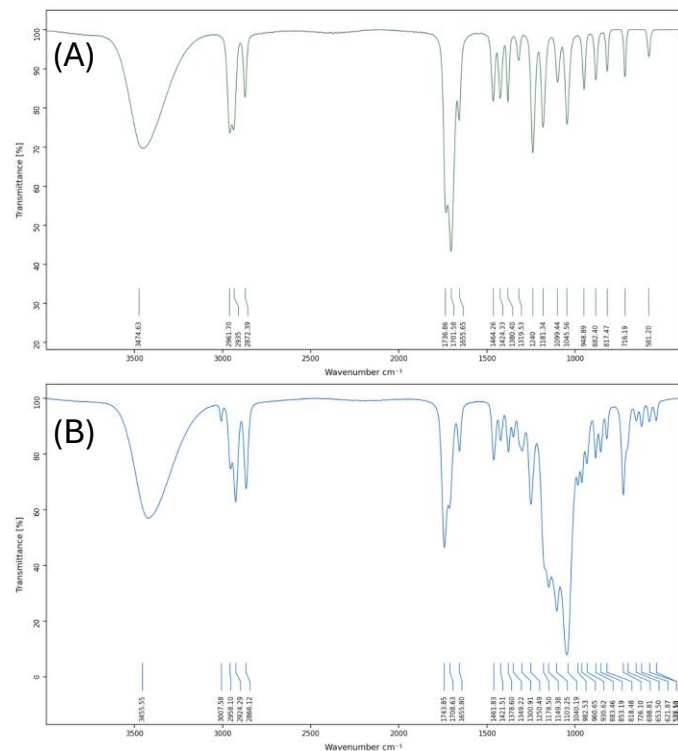


Figure 4: FTIR Spectra of (A) Pure Drug and (B) Physical Mixture

Visual Assessment and Clarity

Upon addition of distilled water, all of the 17 SNEDDS formulations were found to be yellowish, homogeneous and clear with no phase separation in all the batches as shown in Table 4. All the formulations spontaneously formed clear emulsions in 2 minutes which indicated successful self-nanoemulsification for all the compositions of the Box-Behnken design.

Table 4: Visual Assessment and Clarity of SNEDDS Formulations

Batch	Appearance	Homogeneity	Clarity	Phase Separation
F1	Yellowish	Homogeneous	Clear	No phase separation
F2	Yellowish	Homogeneous	Clear	No phase

				separation
F3	Yellowish	Homogeneous	Clear	No phase separation
F4	Yellowish	Homogeneous	Clear	No phase separation
F5	Yellowish	Homogeneous	Clear	No phase separation
F6	Yellowish	Homogeneous	Clear	No phase separation
F7	Yellowish	Homogeneous	Clear	No phase separation
F8	Yellowish	Homogeneous	Clear	No phase separation
F9	Yellowish	Homogeneous	Clear	No phase separation
F10	Yellowish	Homogeneous	Clear	No phase separation
F11	Yellowish	Homogeneous	Clear	No phase separation
F12	Yellowish	Homogeneous	Clear	No phase separation
F13	Yellowish	Homogeneous	Clear	No phase separation
F14	Yellowish	Homogeneous	Clear	No phase separation
F15	Yellowish	Homogeneous	Clear	No phase separation
F16	Yellowish	Homogeneous	Clear	No phase separation
F17	Yellowish	Homogeneous	Clear	No phase separation

Results of Emulsification Time, Percentage Transmittance, Droplet Size and Zeta Potential of SNEDDS Formulations

As presented in Table 5, emulsification time ranged from 43.7 ± 0.7 s (F16) to 85.9 ± 1.4 s (F11), percentage transmittance from $84.6 \pm 0.8\%$ (F11) to $99.1 \pm 0.2\%$ (F16), and droplet size from $101.3 \pm$

1.5 nm (F16) to 198.2 ± 2.9 nm (F11). Zeta potential values ranged from -14.8 ± 0.6 mV (F11) to -25.8 ± 0.6 mV (F6). The formulation F16 showed the most favorable characteristics having minimum droplet size, maximum transmittance and shortest emulsification time.

Table 5: Emulsification Time, Percentage Transmittance, Droplet Size and Zeta Potential of SNEDDS Formulations

Batch	Emulsification Time (s)	Percentage Transmittance (%)	Droplet Size (nm)	Zeta Potential (mV)
F1	55.8 ± 0.9	94.2 ± 0.4	130.8 ± 2.1	-21.3 ± 0.5
F2	56.3 ± 1.0	95.1 ± 0.3	126.4 ± 1.8	-22.1 ± 0.4
F3	52.7 ± 0.8	95.5 ± 0.5	123.2 ± 2.0	-20.2 ± 0.6
F4	77.6 ± 1.2	86.3 ± 0.7	178.9 ± 2.6	-16.8 ± 0.4
F5	44.2 ± 0.7	99.0 ± 0.2	103.7 ± 1.6	-25.4 ± 0.5
F6	46.1 ± 0.9	98.8 ± 0.3	105.4 ± 1.7	-25.8 ± 0.6
F7	55.6 ± 0.8	94.8 ± 0.4	125.7 ± 2.0	-21.9 ± 0.5
F8	70.4 ± 1.1	90.1 ± 0.5	165.2 ± 2.4	-18.6 ± 0.5
F9	54.1 ± 0.9	95.8 ± 0.4	124.1 ± 1.9	-19.8 ± 0.4
F10	69.8 ± 1.0	89.8 ± 0.6	158.6 ± 2.3	-16.4 ± 0.5
F11	85.9 ± 1.4	84.6 ± 0.8	198.2 ± 2.9	-14.8 ± 0.6
F12	56.0 ± 0.9	95.2 ± 0.3	126.9 ± 1.7	-22.3 ± 0.5
F13	55.7 ± 0.8	94.9 ± 0.4	125.9 ± 1.9	-21.8 ± 0.4
F14	63.8 ± 1.1	92.0 ± 0.5	149.3 ± 2.2	-19.0 ± 0.5
F15	56.4 ± 0.9	95.0 ± 0.4	126.2 ± 1.8	-22.0 ± 0.5
F16	43.7 ± 0.7	99.1 ± 0.2	101.3 ± 1.5	-25.2 ± 0.4
F17	64.2 ± 1.0	92.4 ± 0.4	147.9 ± 2.1	-20.4 ± 0.5

pH Measurement, Drug Content Determination and Thermodynamic Stability Studies of SNEDDS

The results presented in Table 6 indicate that all formulations had a pH range between 6.42 ± 0.05 to 7.08 ± 0.04 , which is within the acceptable range for oral administration. The drug content was in the range of $96.36 \pm 0.45\%$ (F11) to $101.58 \pm 0.43\%$ (F16) indicating uniformity of drug distribution within 95–105% acceptance criteria. All 17 formulations performed well in the heating cooling, centrifugation and freeze thaw stress tests to assure thermodynamic stability.

Table 6: pH Measurement, Drug Content Determination and Thermodynamic Stability Studies of SNEDDS Formulations

Batch	pH	Drug Content (%)	Thermodynamic Stability			
			Heating-Cooling Cycle	Centrifugation Test	Freeze-Thaw Cycle	Overall Result
F1	6.78 ± 0.04	99.42 ± 0.38	Passed	Passed	Passed	Stable
F2	6.86 ± 0.03	99.78 ± 0.42	Passed	Passed	Passed	Stable
F3	6.74 ± 0.05	98.96 ± 0.36	Passed	Passed	Passed	Stable
F4	6.48 ± 0.04	97.12 ± 0.41	Passed	Passed	Passed	Stable
F5	7.02 ± 0.03	101.24 ± 0.44	Passed	Passed	Passed	Stable
F6	7.06 ± 0.04	101.46 ± 0.39	Passed	Passed	Passed	Stable
F7	6.85 ± 0.05	99.81 ± 0.35	Passed	Passed	Passed	Stable
F8	6.59 ± 0.04	98.04 ± 0.43	Passed	Passed	Passed	Stable
F9	6.70 ± 0.03	98.52 ± 0.37	Passed	Passed	Passed	Stable
F10	6.54 ± 0.04	97.48 ± 0.40	Passed	Passed	Passed	Stable
F11	6.42 ± 0.05	96.36 ± 0.45	Passed	Passed	Passed	Stable
F12	6.87 ± 0.03	99.74 ± 0.34	Passed	Passed	Passed	Stable

F13	6.84 ± 0.04	99.69 ± 0.38	Passed	Passed	Passed	Stable
F14	6.61 ± 0.05	97.86 ± 0.41	Passed	Passed	Passed	Stable
F15	6.86 ± 0.03	99.76 ± 0.36	Passed	Passed	Passed	Stable
F16	7.08 ± 0.04	101.58 ± 0.43	Passed	Passed	Passed	Stable
F17	6.66 ± 0.05	98.22 ± 0.39	Passed	Passed	Passed	Stable

In Vitro drug dissolution study

The in vitro drug release profiles of all the seventeen SNEDDS formulations (F1–F17) are presented in Figure 5. All the formulations released more than 93% of the drug at 120 minutes and the cumulative release of drug was in the range of $74.92 \pm 0.63\%$ (F11) to $96.10 \pm 0.74\%$ (F16) at 30 minutes. The drug release from the formulations was highest and fastest for formulation F16 and lowest for formulation F11 due to the highest oil concentration. These trends were consistent with the observations about Box-Behnken design, summarized in Table 5.

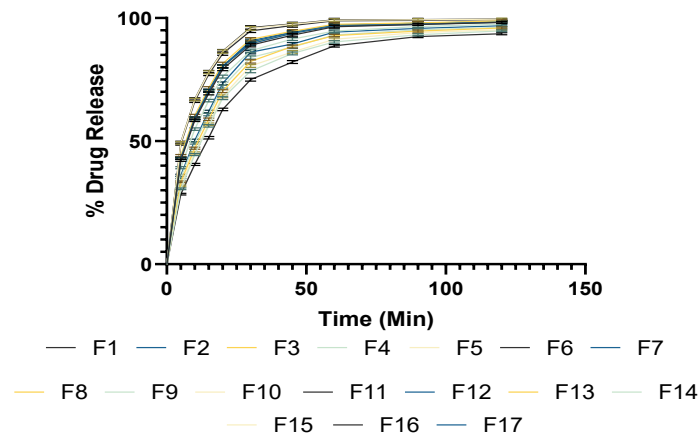


Figure 5: In vitro drug release profile of all batches (F1-F17)

Effect of Formulation Variables on Droplet Size (Y_1)

Droplet size was determined to be best described by the quadratic model as evidenced by the fit summary (Table 8.10) with a model F-value of 6781.81, an adjusted R^2 of 0.9997 and a predicted

R^2 of 0.9993, indicating an excellent model fit. The non-significant lack of fit ($p = 0.6125$) confirmed model fitness. ANOVA (Table 8.11) revealed oil concentration (A) as the most influential factor ($F = 37933.25$, $p < 0.0001$), followed by surfactant (B) ($F = 13590.99$, $p < 0.0001$) and co-surfactant (C) ($F = 3312.39$, $p < 0.0001$). Curvature in the response was confirmed by the presence of all interaction terms (AB, AC, BC) and quadratic terms (A^2 , B^2 , C^2) that were statistically significant ($p < 0.0001$). $Y_1 = 126.22 + 29.74A - 17.80B - 8.79C - 7.48AB + 2.05AC - 4.03BC + 11.69A^2 + 5.57B^2 + 4.24C^2$ Positive coefficient of A (+29.74) showed when the concentration of oil increased, there was a significant increase in droplet size, while the negative coefficient of B (-17.80) and the coefficient of C (-8.79) showed that when the concentration of surfactant and co-surfactant increased, the droplet size decreased significantly. The contour plots (Figure 8.17) showed elliptical patterns for significant interactions between AB, and the 3D response surface plots (Figure 8.18) indicated that the droplet size is strongly related with the higher concentration of oil. The perturbation plot (Figure 8.19) confirmed that oil was the most sensitive variable and the predicted vs. actual plot (Figure 8.20) indicated that there was good agreement between observed and model predicted values.

Effect of Formulation Variables on Cumulative Drug Release at 30 Minutes (Y_2)

The best model was the quadratic model, with an F-value of 98.87 ($p = 1.53 \times 10^{-6}$), adjusted R^2 of

0.9822 and predicted R^2 of 0.8998, which shows that the model is reliable. The non-significant lack of fit ($p = 0.0832$) indicated the model was adequate. ANOVA (Table 8.13) demonstrated that oil concentration (A) exerted the most significant negative effect ($F = 450.31$, $p < 0.0001$), followed by surfactant (B) ($F = 302.62$, $p < 0.0001$) and co-surfactant (C) ($F = 61.79$, $p = 0.0001$). The quadratic terms A^2 , B^2 and C^2 were significant ($p \leq 0.0459$) with the interaction terms AB, AC and BC being non-significant ($p > 0.05$), showing that the relationship between variables and drug release was not linear. $Y_2 = 90.12 - 6.02A + 4.94B + 2.23C + 0.53AB - 0.21AC + 0.62BC - 2.11A^2 - 2.12B^2 - 0.95C^2$ The negative coefficient of oil (-6.02) confirmed the decreased drug release at higher oil concentration while positive coefficients of surfactant (+4.94) and co-surfactant (+2.23) showed an increased drug release with increase in concentration of these components. Contour plots (Figure 8.21) showed curvature indicative of a quadratic effect and 3D response surface plots (Figure 8.22) revealed optimal drug release at higher surfactant and co-surfactant concentrations and lower oil concentrations. The results of the perturbation plot confirmed that the oil and surfactant variables are the most sensitive variables (Figure 8.23), and the predicted vs. actual plot showed that there is a good agreement between the predicted and measured values (Figure 8.24).

Table 7: Fit Summary of Box-Behnken Design for Optimization of Fusidic Acid Loaded SNEDDS

Response	Model	Sequential p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²
Droplet Size (Y ₁)	Quadratic	< 0.0001	0.6125	0.9997	0.9993
Drug Release at 30 min (Y ₂)	Quadratic	0.0005	0.0832	0.9822	0.8998

Table 8: ANOVA Summary for Optimization of Fusidic Acid Loaded SNEDDS

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Droplet Size (Y₁)						
Model	11383.27	9	1264.81	678.18	< 0.0001	Significant
A-Oil	7074.55	1	7074.55	37933.25	< 0.0001	
B-Surfactant	2534.72	1	2534.72	13590.99	< 0.0001	
C-Co-surfactant	617.76	1	617.76	3312.39	< 0.0001	
AB	223.50	1	223.50	1198.40	< 0.0001	
AC	16.81	1	16.81	90.13	< 0.0001	
BC	64.80	1	64.80	347.47	< 0.0001	
A ²	575.39	1	575.39	3085.22	< 0.0001	
B ²	130.40	1	130.40	699.18	< 0.0001	
C ²	75.70	1	75.70	405	<	

				.87	0.0001	
Residual	1.31	7	0.1865			
Lack of Fit	0.4375	3	0.1458	0.6720	0.6125	Not Significant
Pure Error	0.8680	4	0.2170	-	-	-
Cor Total	11384.58	16	-	-	-	-
Cumulative Drug Release at 30 min (Y₂)						
Model	572.91	9	63.66	98.87	< 0.0001	Significant
A-Oil	289.92	1	289.92	450.31	< 0.0001	
B-Surfactant	194.83	1	194.83	302.62	< 0.0001	
C-Co-surfactant	39.78	1	39.78	61.79	0.0001	
AB	1.10	1	1.10	1.71	0.2320	
AC	0.1681	1	0.1681	0.2611	0.6251	
BC	1.51	1	1.51	2.35	0.1692	
A ²	18.70	1	18.70	29.05	0.0010	
B ²	18.88	1	18.88	29.32	0.0010	
C ²	3.78	1	3.78	5.87	0.0459	
Residual	4.51	7	0.6438			
Lack of Fit	3.52	3	1.17	4.75	0.0832	Not Significant
Pure Error	0.9880	4	0.2470			
Cor Total	577.42	16				

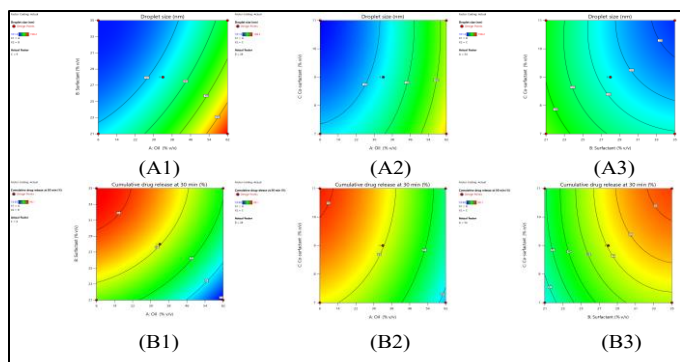


Figure 6: Contour plots illustrating the effect of formulation variables on critical quality attributes of fusidic acid loaded SNEDDS.

(A1 and B1) Effect of oil concentration (X_1) and surfactant concentration (X_2); (A2 and B2) effect of oil concentration (X_1) and co-surfactant concentration (X_3); (A3 and B3) effect of surfactant concentration (X_2) and co-surfactant concentration (X_3) on droplet size (Y_1) and cumulative drug release at 30 minutes (Y_2), respectively.

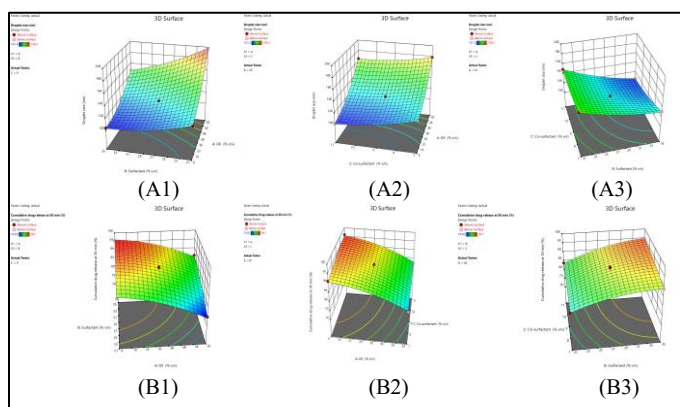


Figure 7: Three-dimensional response surface plots illustrating the effect of formulation variables on critical quality attributes of fusidic acid loaded SNEDDS.

(A1 and B1) Effect of oil concentration (X_1) and surfactant concentration (X_2); (A2 and B2) effect of oil concentration (X_1) and co-surfactant concentration (X_3); (A3 and B3) effect of surfactant concentration (X_2) and co-surfactant concentration

(X_3) on droplet size (Y_1) and cumulative drug release at 30 minutes (Y_2), respectively.

Validation of statistical model

Design-Expert software recommended the optimized formulation F16 which was determined to be the best formulation due to the desirability score of 1.000 shown in Table 9. The experimental values of droplet size and cumulative drug release at 30 minutes (101.3 nm and 96.1%) were well validated with the predicted values (98.914 nm and 96.692%) with low relative errors of 2.41% and 0.61% respectively, indicating good predictability of the developed quadratic model.

Table 9: Validation of statistical model

Batch	Response	Predicted Value	Experimental Value	% Relative error	desirability
F16	Droplet Size	98.914	101.3	2.41	1
	Cumulative drug release at 30 min	96.692	96.1	0.61	

Results of Characterization of Self-Emulsifying Amorphous Solid Dispersion

Solubility of Fusidic acid with different Co-former

The results of the investigations of the solubility of fusidic acid in eight polymers by solvent evaporation method are shown in Table 10. The complete amorphous conversion was obtained for drug:polymer ratio of 1:3 for PVP K30 followed by Soluplus (1:3), PVP K90 and PVP-VA 64 (1:4), HPMC E5 and HPMC E15 (1:5), Eudragit EPO (1:6) and Poloxamer 407 (1:7). Considering the

amorphisation efficiency at minimised polymer concentration, PVP K30 was chosen as an optimal polymer.

Table 10: Solubility of Fusidic Acid in Different Polymers by Solvent Evaporation Method

Sr . N o.	Polym er	Drug:Pol ymer Ratio	Visual Observa tion	Microsc opic Observa tion	Crystalli nity Status
1	PVP K30	1:1	Transpar ent film	Few crystals visible	Partially crystallin e
		1:2	Transpar ent film	Occasion al crystals	Partially crystallin e
		1:3	Transpar ent film	No crystals	Amorph ous
2	PVP K90	1:1	Transpar ent film	Crystals visible	Partially crystallin e
		1:2	Transpar ent film	Few crystals	Partially crystallin e
		1:3	Transpar ent film	Occasion al crystals	Partially crystallin e
		1:4	Transpar ent film	No crystals	Amorph ous
3	HPMC E5	1:1	Transluc ent film	Numero us crystals	Highly crystallin e
		1:2	Transluc ent film	Crystals visible	Partially crystallin e
		1:3	Transluc ent film	Few crystals	Partially crystallin e
		1:4	Transluc ent film	Occasion al crystals	Partially crystallin e
		1:5	Transluc ent film	No crystals	Amorph ous
4	HPMC E15	1:1	Transluc ent film	Numero us crystals	Highly crystallin e
		1:2	Transluc ent film	Crystals visible	Partially crystallin e
		1:3	Transluc ent film	Few crystals	Partially crystallin e
		1:4	Transluc ent film	Occasion al crystals	Partially crystallin e
		1:5	Transluc ent film	No crystals	Amorph ous

5	Solupl us	1:1	Transpar ent film	Crystals visible	Partially crystallin e
		1:2	Transpar ent film	Few crystals	Partially crystallin e
		1:3	Transpar ent film	No crystals	Amorph ous
6	PVP-VA 64	1:1	Transpar ent film	Few crystals visible	Partially crystallin e
		1:2	Transpar ent film	Occasion al crystals	Partially crystallin e
		1:3	Transpar ent film	Occasion al crystals	Partially crystallin e
		1:4	Transpar ent film	No crystals	Amorph ous
7	Eudrag it EPO	1:1	Transluc ent film	Numero us crystals	Highly crystallin e
		1:2	Transluc ent film	Crystals visible	Partially crystallin e
		1:3	Transluc ent film	Crystals visible	Partially crystallin e
		1:4	Transluc ent film	Few crystals	Partially crystallin e
		1:5	Transluc ent film	Occasion al crystals	Partially crystallin e
		1:6	Transluc ent film	No crystals	Amorph ous
8	Poloxa mer 407	1:1	Semi-solid mass	Numero us crystals	Highly crystallin e
		1:2	Semi-solid mass	Crystals visible	Highly crystallin e
		1:3	Semi-solid mass	Crystals visible	Partially crystallin e
		1:4	Semi-solid mass	Few crystals	Partially crystallin e
		1:5	Semi-solid mass	Occasion al crystals	Partially crystallin e
		1:6	Semi-solid mass	Occasion al crystals	Partially crystallin e
		1:7	Semi-solid mass	No crystals	Amorph ous

Drug content and self-emulsification efficiency

The optimized SE-ASD is described in terms of its drug content and characteristics for reconstitution in Table 11. The SE-ASD was found to be $99.12 \pm 0.48\%$ drug loaded with a uniform distribution of the drug throughout the polymer-lipid matrix. Grade A self-emulsification efficiency was seen when the optimized SNEDDS was solidified and it was observed that the self-emulsifying ability of the optimized SNEDDS was retained completely after solidification with rapid formation of clear nanoemulsion.

Table 11: Drug Content Determination and Reconstitution Study of SE-ASD

Batch	Drug Content (%)	Self-emulsification Efficiency	Reconstitution Observation
Optimized SE-ASD	99.12 ± 0.48	Grade A	Rapid self-emulsification with clear nanoemulsion

In vitro Drug Dissolution

The optimized SE-ASD formulation in vitro drug release profile is shown in Figure 8. SE-ASD showed higher drug release rate: at 5 minutes $55.84 \pm 0.52\%$ of the drug was released; at 30 minutes $96.84 \pm 0.81\%$ was released; and at 120 minutes, $99.76 \pm 0.39\%$ of the drug was released. The dissolution rate measured was very fast and indicated the successful amorphous conversion and the self emulsifying property of SE-ASD formulation.

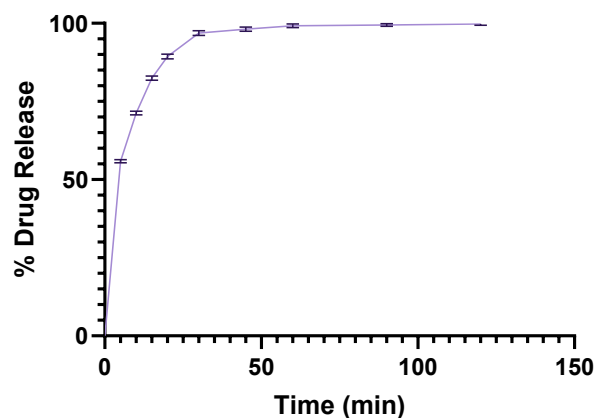


Figure 8: In vitro drug release profile of SE-ASD

Results of XRD study

The diffractograms of PXRD of the pure fusidic acid and optimized SE-ASD are shown in figure 9. Pure fusidic acid (A) gave sharp diffraction peaks at (100), (111), (223), (211), (241) and (253) planes over the range of 2θ , indicating its crystalline nature. The characteristic peaks of (111), (223), and (241) were completely absent, and the other peaks (100), (211), and (253) exhibited significantly reduced intensity and broadened, indicating significant amorphous conversion of fusidic acid in the polymer-lipid matrix for SE-ASD (B).

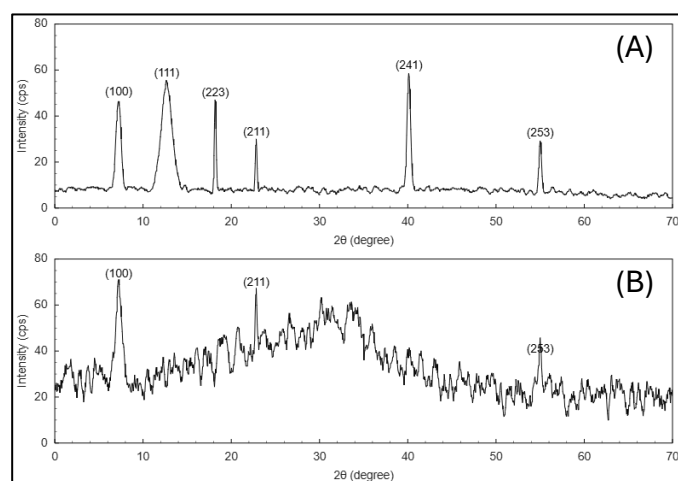


Figure 9: XRD Spectra of (A) Pure Drug and (B) SE-ASD

Results of accelerated stability study

The accelerated stability data for optimized SE-ASD under $40 \pm 2^\circ\text{C}/75 \pm 5\%\text{RH}$ test conditions for 3 months is shown in Table 12. Droplet size showed a marginal increase from 101.3 ± 1.5 nm to 104.9 ± 2.1 nm, while percentage transmittance decreased slightly from $99.1 \pm 0.2\%$ to $98.2 \pm 0.4\%$ and drug content from $101.58 \pm 0.43\%$ to $100.32 \pm 0.56\%$. The percentage drug released at 30 min was found to be above $94.96 \pm 0.65\%$ till the end of the study, suggesting the physicochemical stability of drug release for SE-ASD formulation during the study period.

Table 12: Accelerated Stability Studies of Optimized SNEDDS (F16)

Time (Month)	Droplet Size (nm)	Zeta Potential (mV)	% Transmittance (%)	Emulsification Time (s)	Drug Content (%)	% Drug Release at 30 min
0	101.3 ± 1.5	-25.2 ± 0.4	99.1 ± 0.2	43.7 ± 0.7	101.58 ± 0.43	96.10 ± 0.74
1	102.6 ± 1.7	-24.8 ± 0.5	98.8 ± 0.3	44.2 ± 0.8	101.12 ± 0.48	95.72 ± 0.70
2	103.8 ± 1.9	-24.3 ± 0.5	98.5 ± 0.3	44.9 ± 0.9	100.84 ± 0.52	95.28 ± 0.68
3	104.9 ± 2.1	-23.9 ± 0.6	98.2 ± 0.4	45.6 ± 1.0	100.32 ± 0.56	94.96 ± 0.65

DISCUSSION

Based on the solubility screening data (Table 3), fusidic acid was found to be most soluble in the lipophilic BCS class II solvents such as Transcutol P (269.20 ± 5.32 mg/mL), Tween 80 (135.30 ± 2.45

mg/mL) and soybean oil (15.70 ± 0.42 mg/mL) and this agrees with the reported BCS class II characteristics of fusidic acid [43]. While pseudo-ternary phase diagrams (Figure 2) showed that Smix 3:1 gave the largest self-emulsifying region, this finding is consistent with the results reported by Mahajan et al. on ezetimibe-SNEDDS systems where the presence of higher ratio of surfactant resulted in greater nanoemulsification zone [44]. The DSC thermograms (Figure 3) confirmed that the physical mixture exhibited the drug melting endotherm peak at 192.54°C which is in accordance with the finding that there was no significant shift in the drug melting endotherm peak and this result is in agreement with previously reported results of compatibility that were observed in SNEDDS systems [45]. FTIR analysis (Figure 4) showed that all characteristic functional groups peaks were retained in the physical mixture, which further confirmed the physicochemical compatibility of the drug and excipients. The BBD optimization using Design-Expert software gave a quadratic model with good predictability for the droplet size ($\text{Adj } R^2 = 0.9997$) and drug release at 30 minutes ($\text{Adj } R^2 = 0.9822$) (Tables 7–8), and oil concentration was the most significant variable ($F = 37933.25$, $p < 0.0001$), as reported by Patel et al., who found the same variable to be the most significant in the quadratic model for droplet size of BBD-optimized SNEDDS [45]. Table 5 shows that optimized formulation F16 gave droplet size of 101.3 ± 1.5 nm, percentage transmittance of $99.1 \pm 0.2\%$ and emulsification time of 43.7 ± 0.7 s with cumulative

drug release of $96.10 \pm 0.74\%$ at 30 minutes, which is considered to be good and confirmed by its desirability score of 1.000 and low relative prediction error (Table 9) after model validation [46].

In the screening of polymers for SE-ASD development, the polymer PVP K30 was found to be the optimal carrier, where complete amorphous conversion was obtained with the lowest drug:polymer ratio 1:3, in accordance with previous reports of the ability of PVP K30 to act as an efficient crystallization inhibitor and hydrogen bond donor in the preparation of ASDs [47]. The complete disappearance of the crystalline diffraction planes (111), (223) and (241) of fusidic acid in the SE-ASD and the marked broadening and intensity reduction of the remaining peaks confirmed complete amorphization of the crystalline material, which is consistent with other PXRD reported amorphization of glibenclamide SE-ASD systems [48]. The SE-ASD exhibited significantly higher drug dissolution ($96.84 \pm 0.81\%$) at 30 minutes than those of the conventional drug formulations previously reported (Figure 8), which was attributed to the synergy between the drug being amorphous and the spontaneous nanoemulsification upon contact with water, as evidenced by studies on solid SNEDDS reporting up to 19 fold improvement in dissolution efficiency compared to crystalline drug [48]. The results of accelerated stability studies at $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$ for 3 months (Table 12) showed that the droplet size (101.3 to 104.9 nm), the drug content (101.58 to 100.32%) and the drug

release (96.10 to 94.96%) were close to the acceptance criteria of ICH Q1A(R2), demonstrating the physicochemical stability of the SE-ASD [49]. The combined results show that SE-ASD is a solid and stable system for increasing the oral bioavailability of fusidic acid, as the polymer-lipid matrix proved to be effective in preventing the recrystallization of the drug and still maintaining self-emulsification properties, consistent with those previously reported for hybrid ASD-SNEDDS systems compared with conventional lipid-based formulations [50].

CONCLUSION

The current study has successfully developed and characterized self-emulsifying amorphous solid dispersion of fusidic acid by optimizing using a systematic Box-Behnken design approach. The optimized formulation F16 (PVP K30: drug ratio of 1:3) showed a nanometric droplet size ($101.3 \pm 1.5 \text{ nm}$), excellent drug release percentage (96.84% after 30 minutes) and proved to be amorphous by PXRD. Physicochemical stability was confirmed by accelerated stability studies, which showed stability for three months under ICH specified conditions. The SE-ASD platform solves critical biopharmaceutical challenges of fusidic acid, such as low aqueous solubility, variable bioavailability and subtherapeutic oral bioavailability that have hindered its clinical application for treating staphylococcal infections caused by MRSA. In further studies, in vivo pharmacokinetic studies, ex vivo permeation studies, and evaluation of antimicrobial effectiveness in animal models are warranted to prove the superiority of the developed

SE-ASD formulation from a therapeutic point of view.

ABBREVIATIONS

SE-ASD: Self-Emulsifying Amorphous Solid Dispersion; SNEDDS: Self-Nanoemulsifying Drug Delivery System; BCS: Biopharmaceutical Classification System; BBD: Box-Behnken Design; MRSA: Methicillin-Resistant *Staphylococcus aureus*; DSC: Differential Scanning Calorimetry; FTIR: Fourier Transform Infrared Spectroscopy; PXRD: Powder X-Ray Diffraction; ANOVA: Analysis of Variance; RSM: Response Surface Methodology; ICH: International Council for Harmonisation; PVP: Polyvinylpyrrolidone; HPMC: Hydroxypropyl Methylcellulose; UV: Ultraviolet Spectrophotometry; PDI: Polydispersity Index; rpm: Revolutions Per Minute; SD: Standard Deviation; DoE: Design of Experiments; Smix: Surfactant-to-Co-surfactant Mixture Ratio; ASD: Amorphous Solid Dispersion; SEDDS: Self-Emulsifying Drug Delivery System; GI: Gastrointestinal; EF-G: Elongation Factor G; AMR: Antimicrobial Resistance.

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