# Design And Evaluation Of A Self-Nanoemulsifying Drug Delivery System For Improved Topical Delivery Of 5-Fluorouracil In Actinic Keratosis

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**Key words:** Fluorouracil; Keratolysis, Chemical; Drug Delivery Systems / methods; Emulsions; Administration, Topical.

**Abstract**

**Background:** Topical 5-Fluorouracil (5-FU) is a standard therapy for actinic keratosis (AK), yet its clinical use is often restricted by inadequate skin penetration and dose-related irritation from conventional formulations. Formulating 5-FU as a Self-Nanoemulsifying Drug Delivery System (SNEDDS) may improve drug delivery efficiency and minimize adverse reactions.

**Methods:** Preformulation compatibility between 5-FU and excipients was investigated through Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), and X-ray Diffraction (XRD) analysis. Using a Central Composite Design (CCD), a SNEDDS containing isopropyl myristate, Tween 80, and Transcutol P was optimized. The selected formulation (F2) underwent characterization for particle size, zeta potential, polydispersity index (PDI), drug content, and entrapment efficiency. This optimized SNEDDS was incorporated into a cream (CF2) and assessed for spreadability, drug release, and in vitro cytotoxicity against A549 skin carcinoma cells via the MTT assay.

**Results:** Analytical studies confirmed the absence of drug–excipient incompatibility. The optimized SNEDDS (F2) exhibited a particle size of 178.8 nm, zeta potential of −20 mV, PDI of 0.828, drug content of 88.19 %, and entrapment efficiency of 87.13 %. The SNEDDS-loaded cream (CF2) demonstrated excellent spreadability, drug release (88.67 %), and entrapment efficiency (90.13 %). Cytotoxic evaluation revealed an $IC_{50}$ value of 39.53 µg/mL, indicating comparable efficacy to pure 5-FU.

**Conclusion:** Incorporating 5-FU into a SNEDDS-based cream improved its topical delivery and anticancer activity, suggesting its potential as an effective and patient-compliant therapy for precancerous skin conditions.

## 1. Introduction

Actinic keratosis (AK) is a common pre-malignant skin condition that arises due to cumulative exposure to ultraviolet radiation, leading to dysplastic changes in keratinocytes. If untreated, AK may progress into squamous cell carcinoma.[1] 5-Fluorouracil (5-FU), a pyrimidine analog, is widely used in the topical treatment of AK due to its potent antiproliferative action.[2] However, its hydrophilic nature and

low lipophilicity limit its penetration through the stratum corneum, necessitating high concentrations in formulations that may cause irritation and reduce patient compliance.[3]

To address these limitations, Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) offer a viable strategy. SNEDDS are isotropic mixtures of oils, surfactants, and co-surfactants that spontaneously form fine oil-in-water nanoemulsions upon contact with aqueous environments such as skin moisture.[4][5][6] These systems can enhance drug solubility, improve dermal penetration, and enable sustained drug release. This study focuses on formulating a SNEDDS-based cream for enhanced topical delivery of 5-FU using a Quality by Design (QbD) approach.[7]

## 2. Materials and Methods

### 2.1 Materials

5-Fluorouracil and pharmaceutical-grade excipients including Isopropyl Myristate (IPM), Tween 80, and Transcutol P were procured from certified suppliers. All reagents were of analytical grade and used as received.

### 2.2 Preformulation Studies

The solubility of 5-FU was evaluated in various oils, emulsification ability of different surfactants, and co-surfactants was evaluated.[8] Isopropyl myristate showed the highest solubility among oils (49.32 mg/mL), Tween 80 among surfactants (72.27 mg/mL), and Transcutol P among co-surfactants (65.71 mg/mL). FTIR, DSC, and XRD analyses were conducted to assess the compatibility of 5-FU with selected excipients.[9]

### 2.3 Formulation Optimization Using CCD

Central Composite Design (CCD) was applied via Design-Expert® 13 software to optimize the SNEDDS formulation. Independent variables included concentrations of oil, surfactant, and co-surfactant, while responses were drug content, entrapment efficiency, and in vitro drug release. Seventeen experimental runs were generated.

### 2.4 Characterization of SNEDDS

Optimized SNEDDS formulations were evaluated for emulsification time, particle size, zeta potential, and polydispersity index (PDI). Morphological analysis was carried out using Scanning Electron Microscopy (SEM). Drug content and entrapment efficiency were determined using validated UV-spectrophotometric methods.[10]

### 2.5 SNEDDS Cream Formulation and Evaluation

The optimized SNEDDS (F2) was incorporated into a cream base containing stearic acid, cetylstearyl alcohol, oleic acid, and HPMC K15. The resultant cream (CF2) was evaluated for physical appearance, pH, viscosity, spreadability, drug content, entrapment efficiency, and in vitro diffusion using Franz diffusion cells.[11]

### 2.6 In Vitro Cytotoxicity (MTT Assay)

The cytotoxicity of CF2 was tested on A549 human skin cancer cells using the MTT assay. The $IC_{50}$ value of CF2 was compared with that of standard 5-FU solution.

### 2.7 Stability Studies

Stability of the cream formulation was studied under accelerated conditions ($40 \pm 2°C/75 \pm 5\%$ RH) for three months following ICH Q1A(R2) guidelines. Periodic assessments included drug content, viscosity, and physical changes.

## 3. Results and Discussion

3.1 Preformulation Studies

The physical and chemical properties of 5-Fluorouracil (5-FU) were confirmed through comprehensive preformulation studies. The drug appeared as white, crystalline powder with a sharp melting point at 282°C. It was sparingly soluble in water and PBS pH 7.4 but showed good solubility in methanol. The ultraviolet spectrophotometric analysis revealed a λmax at 266 nm. FTIR spectroscopy displayed characteristic peaks corresponding to major functional groups of 5-FU without any observable shifts when mixed with excipients, indicating compatibility. Furthermore, X-ray diffraction patterns exhibited intense and sharp peaks, validating the crystalline nature of the drug.

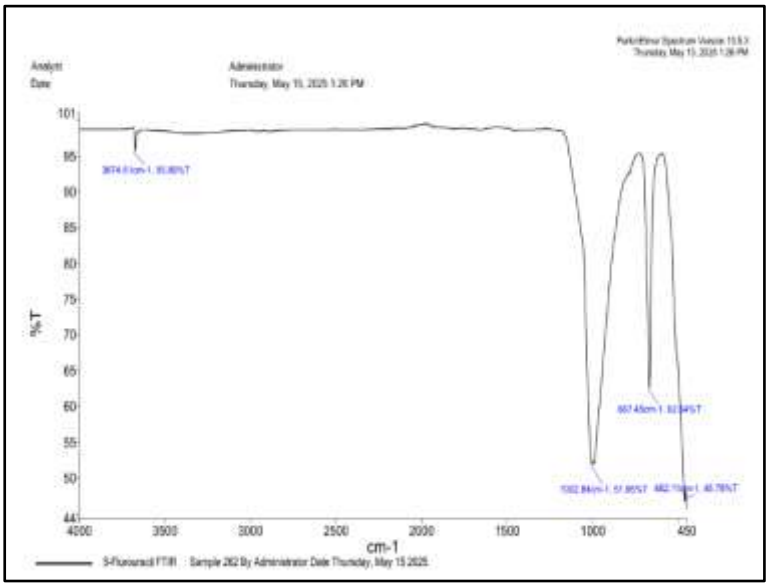


**Fig. 3.1: FTIR of pure drug**

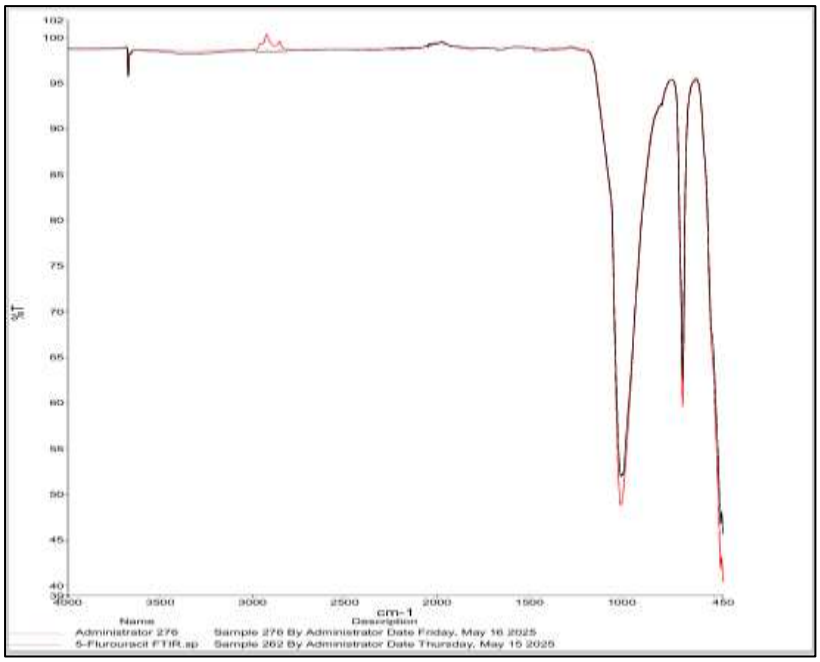


**fig. 3.2: Graph showing compatibility results of drug with excipients**

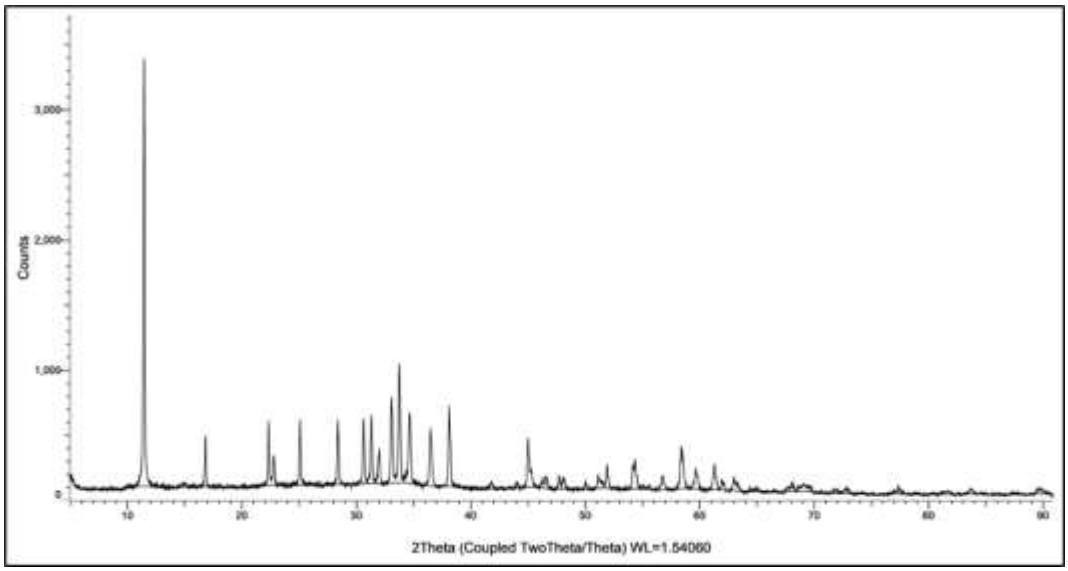


**Fig. 3.3: XRD of pure drug**

3.2 Solubility and Excipient Screening

Solubility studies conducted using various oils, surfactants, and co-surfactants identified Isopropyl Myristate (IPM) as the most suitable oil phase, showing maximum solubility for 5-FU. Among surfactants, Tween 80 showed the highest emulsification efficiency, and Transcutol P was chosen as the optimal co-surfactant due to its strong co-emulsification ability. These excipients were selected based on their ability to solubilize the drug efficiently while maintaining emulsion clarity and stability.

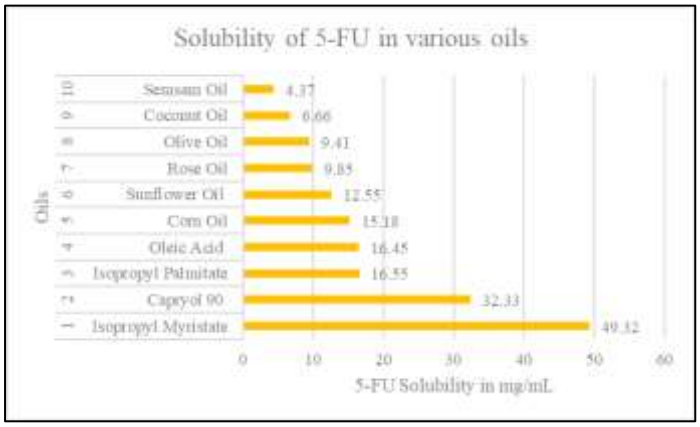


**Fig. 3.4: solubility of 5-FU in various oils**

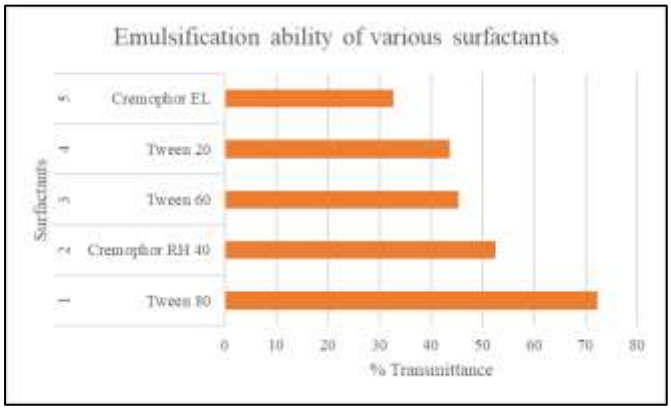


**Fig. 3.5: emulsification ability of various surfactants**

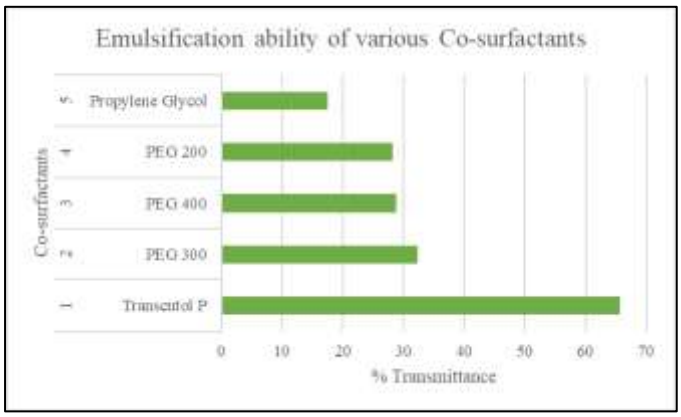


**Fig. 3.6: Emulsification ability of various co-surfactants**

3.3 Optimization Using Central Composite Design (CCD)

The formulation of 5-Fluorouracil-loaded SNEDDS-based topical cream was statistically optimized using Central Composite Design (CCD) with the aid of Design-Expert® software (Version 13.0.5). The design included three independent formulation variables: A – Oil concentration (mL), B – Surfactant concentration (mL), and C – Co-surfactant concentration (mL). The corresponding dependent responses studied were: Drug Content ($Y_1$, %), Entrapment Efficiency ($Y_2$, %), and Drug Release ($Y_3$, %).

A total of 17 experimental runs were generated. Among the different models evaluated (linear, 2FI, and quadratic), the linear model was identified as the most suitable based on ANOVA results, where it demonstrated statistical significance ($p < 0.05$) and the best fit with acceptable levels of lack-of-fit and model adequacy. The 2FI and quadratic models were rejected due to poor fit and insignificant p-values ($p > 0.05$).

The final equations generated for each response, in terms of coded variables, were:

Drug Content ($Y_1$):

$Y_1 = 78.35 + 3.48A – 1.82B – 1.01*C$

Entrapment Efficiency ($Y_2$):

$Y_2 = 75.28 + 3.77A – 2.95B – 1.84*C$

Drug Release ($Y_3$):

$Y_3 = 75.29 + 3.12A – 2.49B – 2.20*C$

These equations suggest that oil concentration (Factor A) had a significant positive effect on all three responses, whereas surfactant (B) and co-surfactant (C) showed inverse relationships. The 3D response surface plots (Figure 3.8) clearly illustrated the influence of formulation variables on each response parameter.

Based on the desirability function approach, the optimized formulation (Batch F2) consisted of 20 mL oil, 20 mL surfactant, and 10 mL co-surfactant, resulting in 88.19% drug content, 87.13% entrapment efficiency, and 85.65% drug release. These experimental results closely matched the model predictions, confirming the reliability and predictive ability of the applied CCD model.

**Table 3.1: CCD table**

| | **Factor 1** | **Factor 2** | **Factor 3** | **Response 1** | **Response 2** | **Response 3** |
|---|---|---|---|---|---|---|
| **Run** | **A: oil** | **B: surfactant** | **C: co-surfactant** | **Drug Content** | **Entrapment Efficiency** | **Drug Release** |
| | mL | mL | mL | % | % | % |
| 1 | 20 | 30 | 8 | 74.32 | 73.42 | 75.12 |
| **2** | **20** | **20** | **10** | **88.19** | **87.13** | **85.65** |
| 3 | 15 | 16.59 | 10 | 79.26 | 77.05 | 77.57 |
| 4 | 15 | 25 | 10 | 71.14 | 70.36 | 69.4 |
| 5 | 20 | 30 | 12 | 82.57 | 80.77 | 82.02 |
| 6 | 10 | 30 | 12 | 77.89 | 75.64 | 75.93 |
| 7 | 15 | 33.41 | 10 | 73.52 | 67.63 | 67.77 |
| 8 | 15 | 25 | 10 | 70.05 | 63.47 | 63.69 |
| 9 | 10 | 30 | 8 | 82.73 | 79.32 | 78.38 |
| 10 | 10 | 20 | 8 | 70.84 | 65.64 | 66.95 |
| 11 | 23.41 | 25 | 10 | 81.44 | 81.93 | 79.2 |
| 12 | 15 | 25 | 6.64 | 71.22 | 67.59 | 65.32 |
| 13 | 10 | 20 | 12 | 69.86 | 64.25 | 64.5 |
| 14 | 15 | 25 | 13.36 | 76.13 | 76.31 | 74.3 |
| 15 | 6.59 | 25 | 10 | 73.69 | 71.48 | 71.04 |
| 16 | 20 | 20 | 12 | 75.32 | 72.96 | 72.67 |
| 17 | 15 | 25 | 10 | 78.03 | 77.18 | 76.75 |

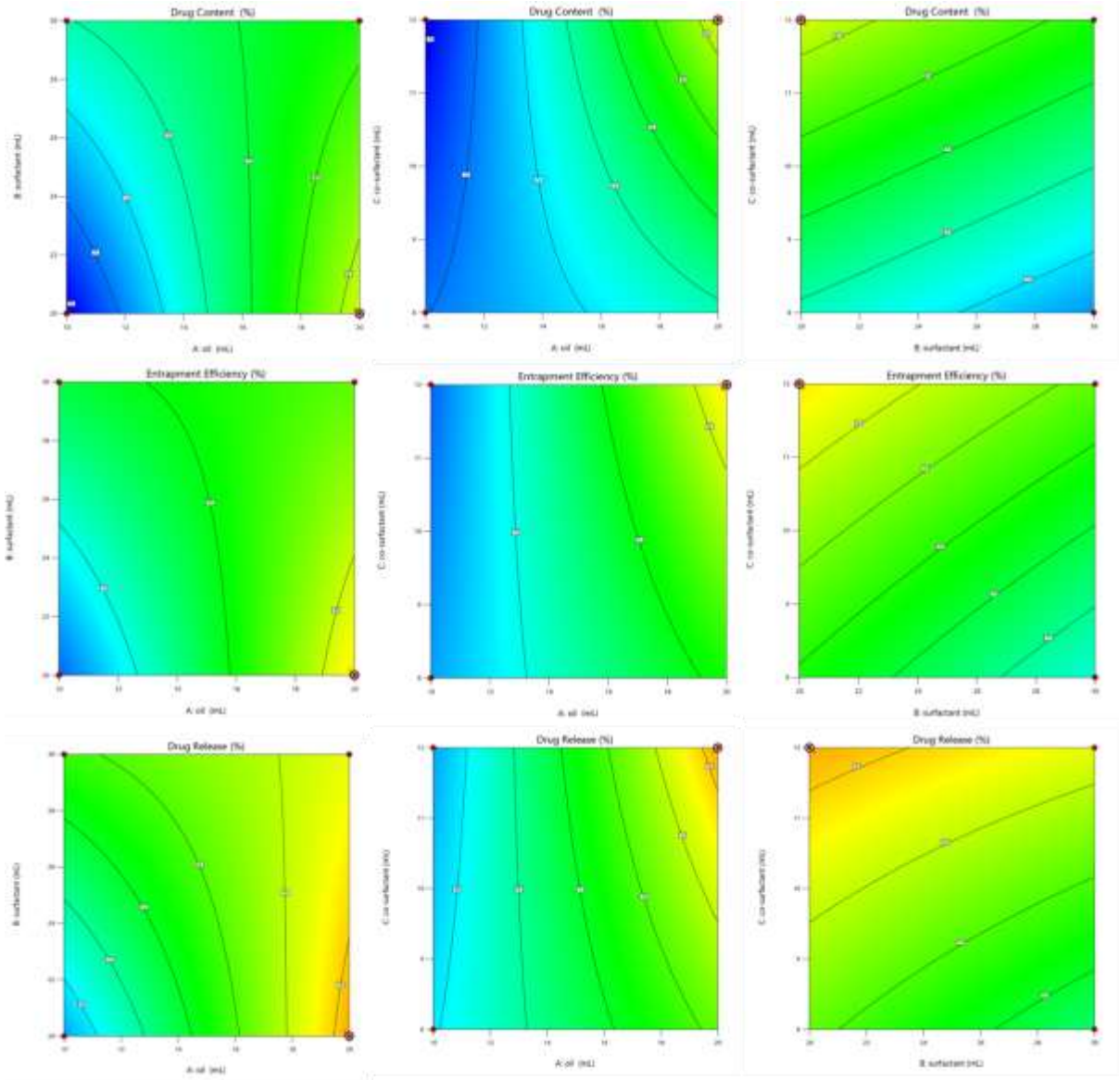


**Fig. 3.7: 2D Contour Plot graphs showing: influence of oil, surfactant and co-surfactant on (a) % drug content ;(b) % entrapment efficiency; (c) % drug release.**

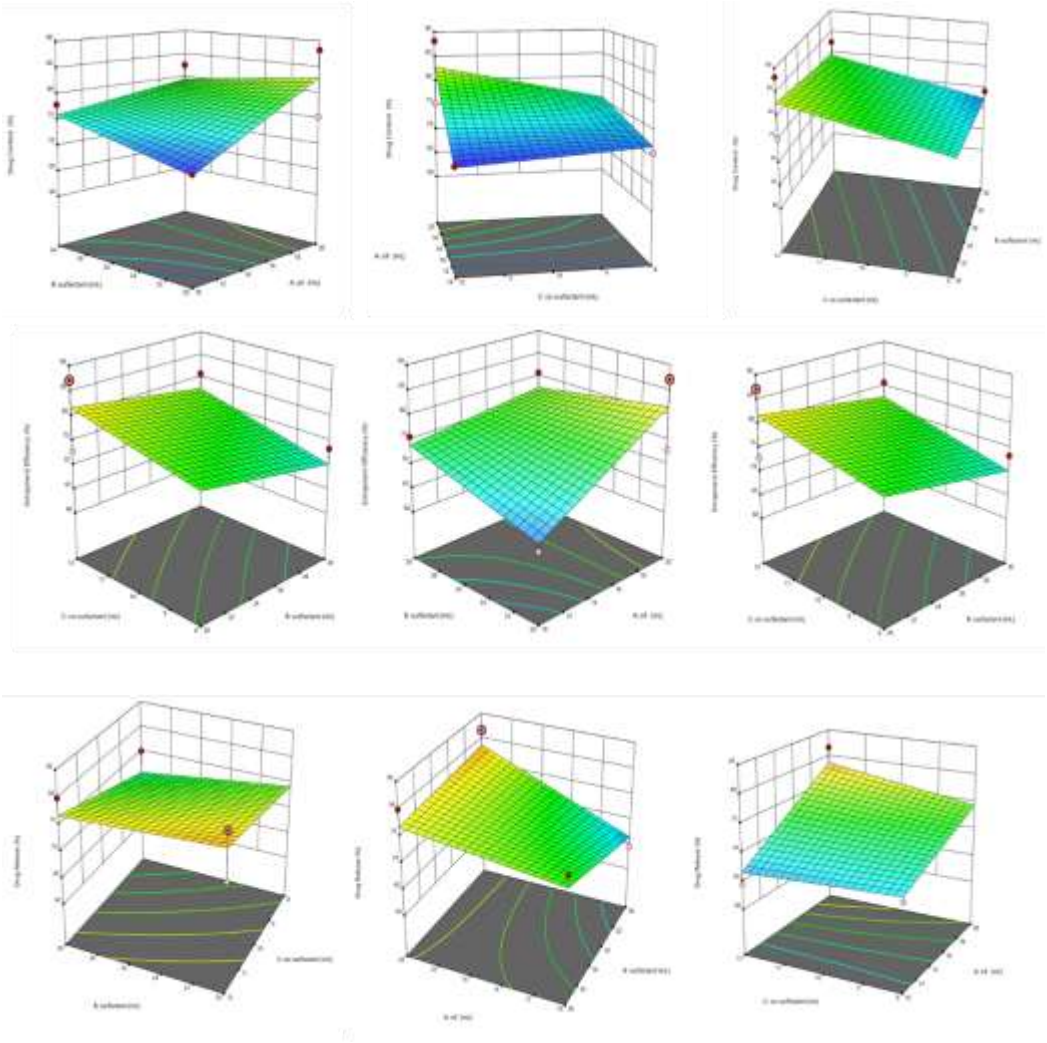

**Fig. 3.8: 3D surface graphs showing: influence of oil, surfactant and co-surfactant on (a) % drug content ;(b) % entrapment efficiency; (c) % drug release.**

3.4 Evaluation of SNEDDS Formulation

The optimized SNEDDS formulation (F2) was clear, isotropic, and stable. It showed a rapid emulsification time of 3.2 seconds upon dilution with aqueous media. Particle size analysis revealed a mean globule diameter of 178.8 nm with a polydispersity index (PDI) of 0.828, indicating uniform droplet distribution. The zeta potential was recorded at −20 mV, suggesting electrostatic stability of the nanoemulsion. Microscopy and SEM analyses confirmed the spherical shape and smooth surface of the nanoemulsion droplets.

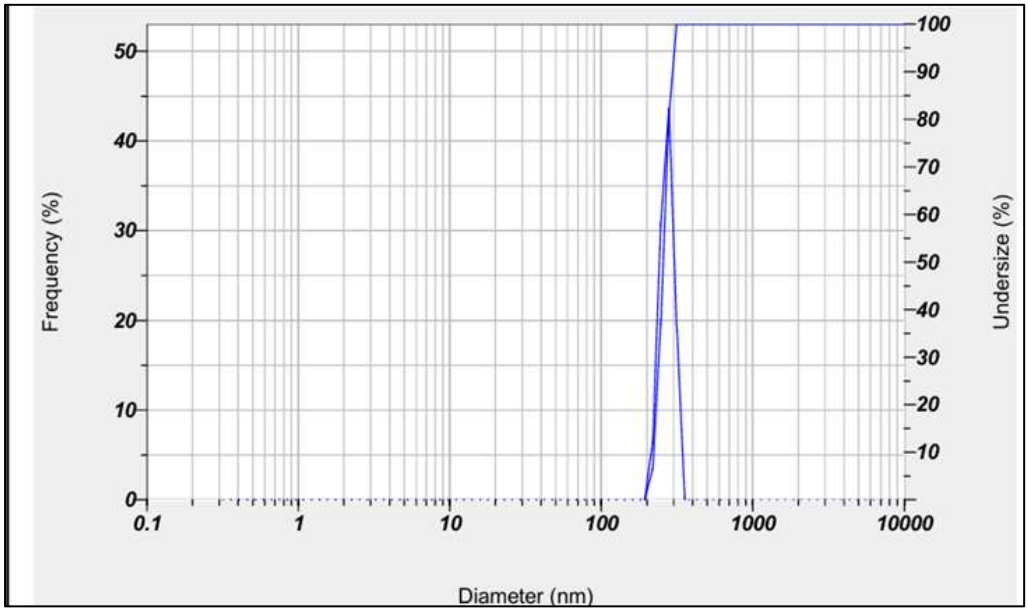


**Fig. 3.9: Particle Size of SNEDDS**

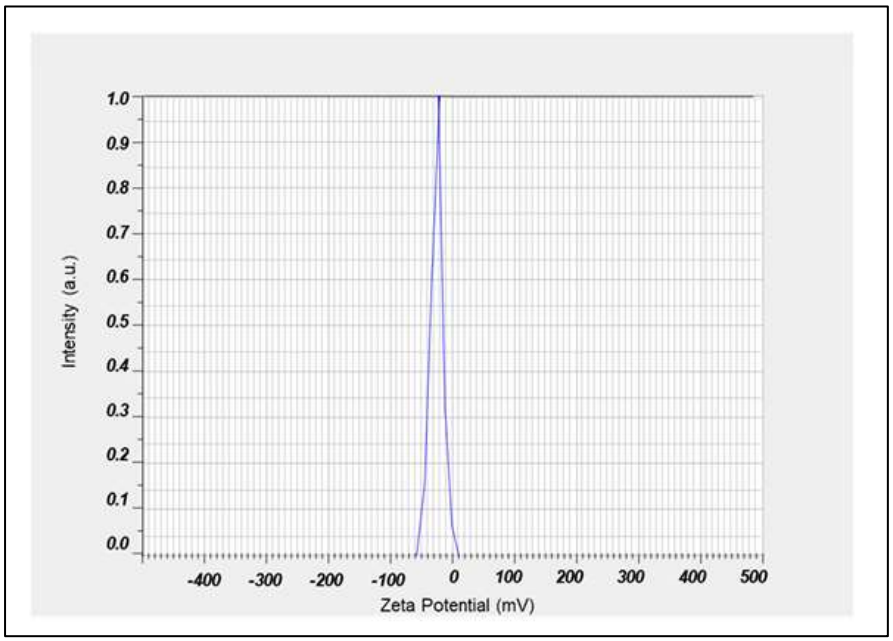


**Fig. 3.10: Zeta potential of SNEDDS**

**Table no. 3.2: particle size, PDI and zeta potential of SNEDDS**

| Formulation | Particle size | PDI | Zeta potential |
|---|---|---|---|
| F2 | 178.8nm | 0.828 | -20mV |

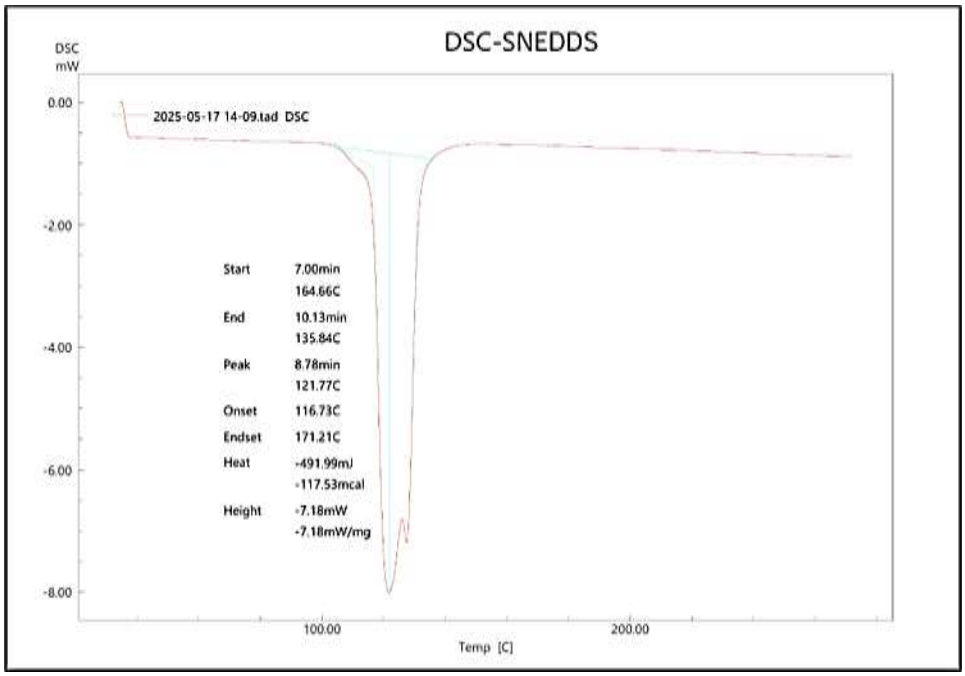


**Fig. 3.11: DSC of SNEDDS**

3.5 Drug Content and Entrapment Efficiency

All formulations were evaluated for their drug content and entrapment efficiency. F2 batch displayed the highest drug content of 88.19% and entrapment efficiency of 87.13%, confirming efficient incorporation of 5-FU into the lipid phase. These results validated the solubilizing ability of the selected excipients and the stability of the drug within the SNEDDS matrix.

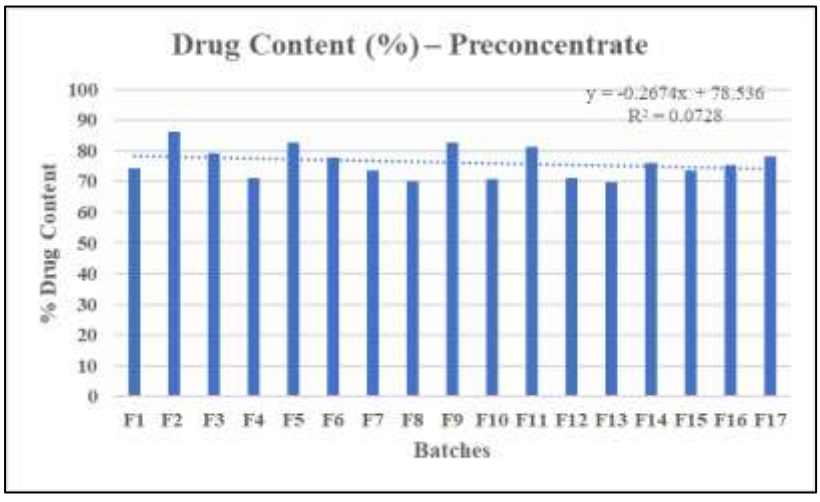


**Fig. 3.12: % Drug Content of all SNEDDS**

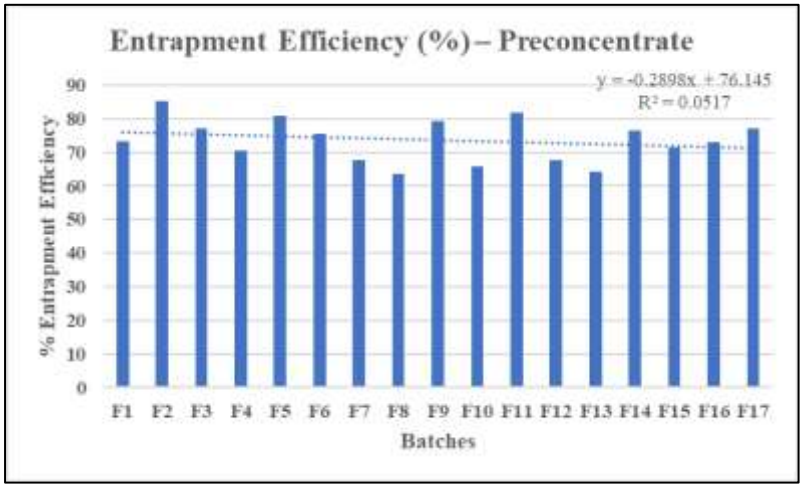


**Fig. 3.13: % Entrapment efficiency of all SNEDDS**

3.6 In Vitro Drug Diffusion of SNEDDS

In vitro diffusion studies of the SNEDDS formulations were performed using Franz diffusion cells. The F2 formulation showed the maximum cumulative drug release of 85.65% over a 14-hour period, reflecting sustained release characteristics. The drug release followed Higuchi kinetics, suggesting diffusion-controlled release from the lipid matrix.

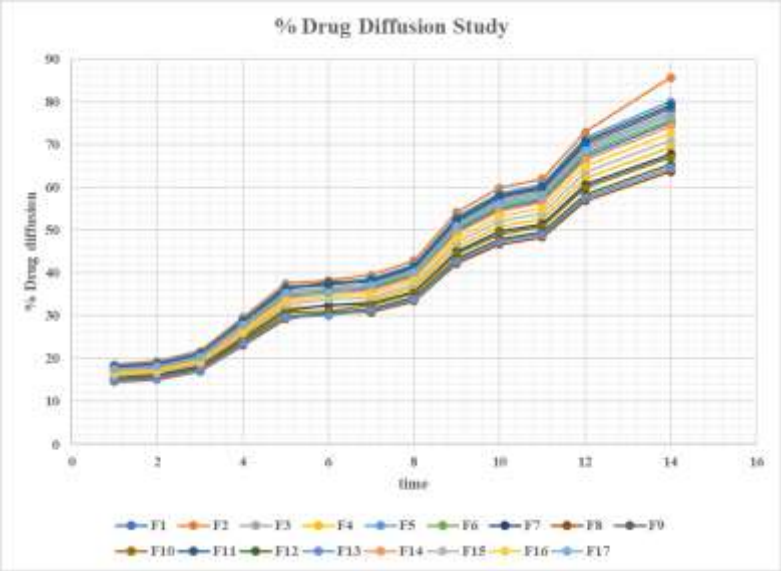


**Fig. 3.14: % In-Vitro drug diffusion of all SNEDDS**

3.7 Stability Studies of SNEDDS

Stability evaluation of the F2 SNEDDS under accelerated storage conditions showed no significant change in physical appearance, pH, or drug content over 30 days. The formulation remained stable without phase separation or drug precipitation, supporting its robustness.

3.8 Evaluation of SNEDDS-Based Cream Formulations

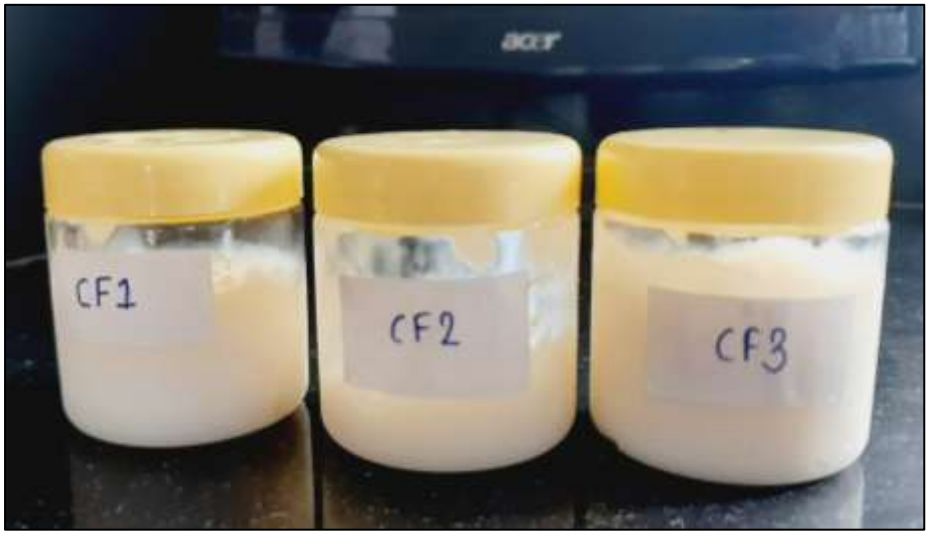


**Fig. 3.15: cream formulations**

**Fig. 3.16: Cf2 cream**

The optimized SNEDDS formulation (F2) was incorporated into a cream base to develop a topical delivery system. Among the prepared creams (CF1–CF3), CF2 showed the most desirable characteristics. It exhibited a pH of 5.57, closely matching the skin's natural pH. The viscosity was measured at 23,000 cP, providing suitable consistency and spreadability. Spreadability of CF2 was found to be 6.92 g·cm/s, indicating ease of application on the skin surface. Drug content was observed to be 91.80%.

**Table 3.3: Appearance, pH, viscosity and spreadability**

| Cream formulation | Appearance | pH | Viscosity (cP) | Spreadability (g·cm/sec) |
|---|---|---|---|---|
| CF-1 | Smooth, creamy | 5.56 | 22,900 | 6.81 |
| CF-2 | | 5.57 | 23,000 | 6.92 |
| CF-3 | | 5.57 | 23,600 | 6.73 |

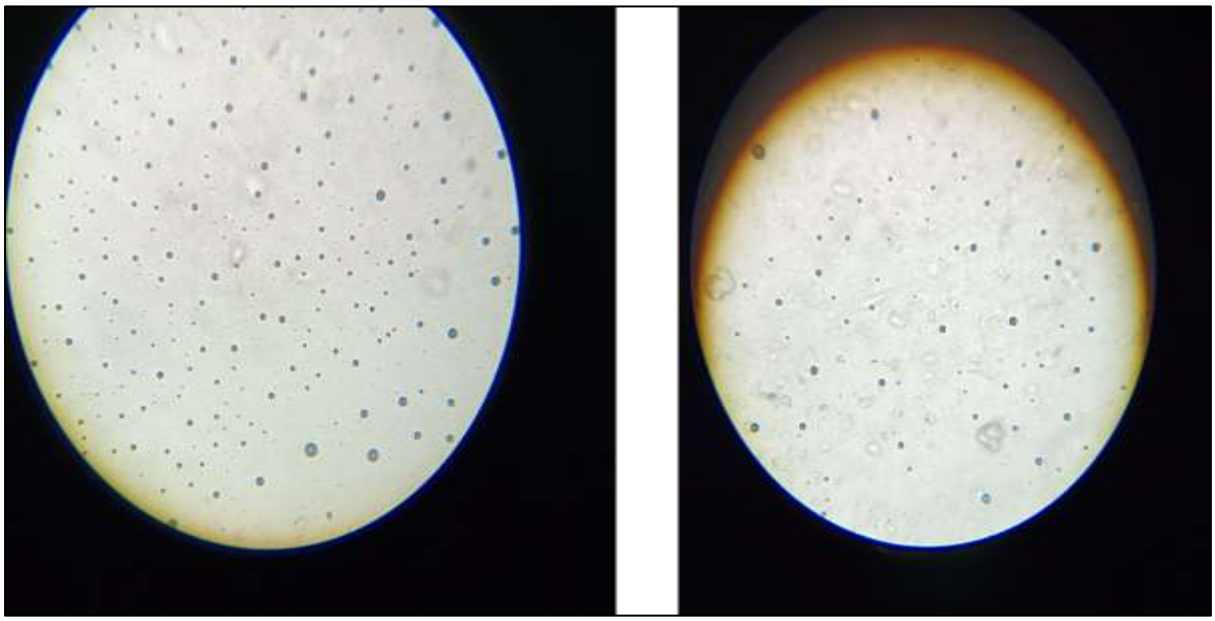

**Fig. 3.17: Microscopic images of cream**

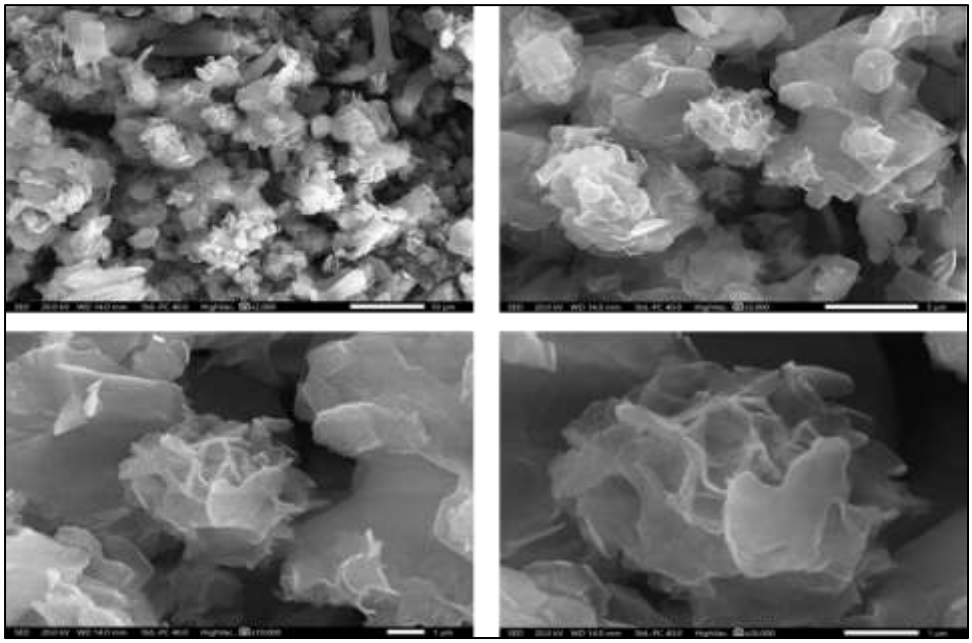

**Fig. 3.18: SEM images of cream**

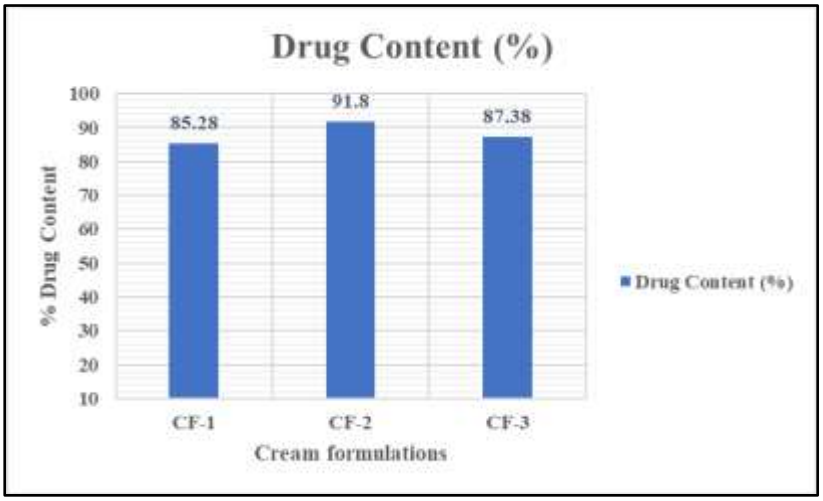


**Fig. 3.19: % Drug content of creams**

3.9 In Vitro Drug Release from SNEDDS Cream

The in vitro release profile of CF2 cream showed sustained drug diffusion with 88.67% of 5-FU released over 14 hours. The results suggested a controlled release pattern suitable for prolonged therapeutic action on the skin. When compared with the plain SNEDDS formulation, the cream matrix slightly delayed the release, which is advantageous for topical retention.

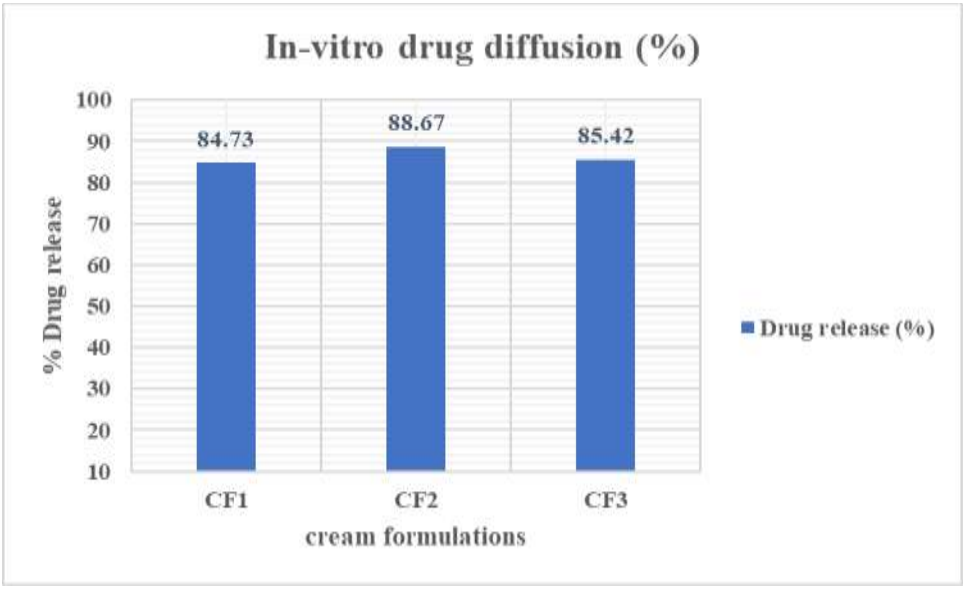


**Fig. 3.20: % In-Vitro drug diffusion of creams**

3.10 Stability Study of SNEDDS Cream

Accelerated stability testing of CF2 cream over three months revealed minimal changes in viscosity, pH, and drug content. Drug diffusion remained above 87%, indicating excellent formulation stability and integrity of the SNEDDS-loaded cream.

3.11 MTT Assay and Anticancer Activity

The cytotoxic potential of the optimized cream (CF2) was evaluated on A549 skin carcinoma cells using the MTT assay. The $IC_{50}$ value of CF2 was calculated to be 39.53 µg/mL, closely matching the standard

5-FU solution ($IC_{50}$ = 38.12 µg/mL). This result confirmed that 5-FU retained its anticancer efficacy within the SNEDDS-based topical formulation and remained biologically active after formulation processing.

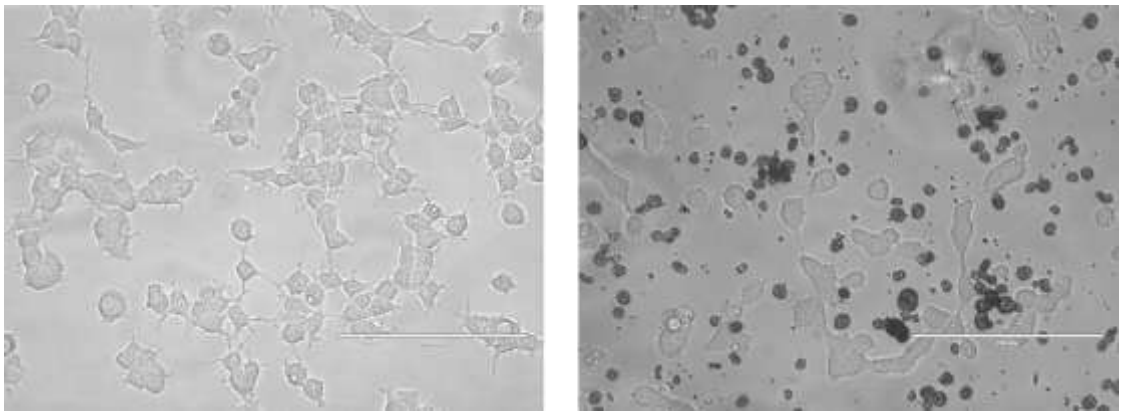

**Fig. 3.21: Comparison between Control and CF2**

## 4. Conclusion:

The present study successfully developed a SNEDDS-based cream formulation for topical delivery of 5-FU with improved permeability and therapeutic potential. The formulation significantly enhanced drug retention in the skin, provided controlled release, and demonstrated cytotoxicity against cancer cells, supporting its potential use in treating actinic keratosis. This approach could be a valuable alternative to conventional high-dose topical therapies and aligns with current advancements in nanotechnology-based drug delivery.

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Conflicts of Interest:

The authors declare no conflict of interest.

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