**RESEARCH PAPER**

# Solid Self Nanoemulsifying Drug Delivery System of Cariprazine: Formulation Insights, Optimization, Cell Viability Assessment and Bioavailability Study

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**ABSTRACT**

**Purpose:** The objective of the present study was to prepare a solid self-nano emulsifying drug delivery system (S-SNEDDS) of Cariprazine Hydrochloride (CPH), a poorly water-soluble drug used in schizophrenia.

**Methods:** Equilibrium Solubility study and ternary phase diagram study were carried out for screening of oils and mixture of surfactants and co-surfactants. D-optimal Mixture design was utilised to optimise the independent variables, X1 (amount of oil; Capmul MCM C8), X2 (amount of surfactant; Tween 80) and X3 (amount of co-surfactant; PEG-300). Self-emulsification time (Y1), percentage transmittance (Y2) and average globule size (Y3) were set as dependent variables. Optimized liquid SNEDSS (L-SNEDDS) formulation was characterized for robustness to dilution, thermodynamic stability, cell line study and TEM. L-SNEDDS was converted into solid SNEDDS (S-SNEDDS) by adsorption technique on the porous carrier like Syloid XDP. S-SNEDDS further filled in to hard gelatin capsules (HGC) and evaluated for in-vitro drug release, stability and bioavailability study.

**Results:** The optimized L-SNEDDS formulation consists of 20% oil, 40% surfactant, and 40% co-surfactant, demonstrating strong thermodynamic stability and safety for cellular use.TEM analysis demonstrated that the nanoemulsion comprised spherical, uniformly sized globules. When testing the in vitro dissolution of HGC prepared from CPH-loaded S-SNEDDS, there was a tremendous increase in the drug release, achieving 98% within 30 min. Further stability testing as per ICH guidelines for a six-month period indicated that the HGC remained stable, with no significant changes in its physical characteristics. The bioavailability study revealed a 1.6-fold increase in the relative bioavailability of S-SNEDDS as compared to the active medicament alone.

**Conclusion:** It has been observed that S-SNEDDS has the ability to create a nanosize dispersion and enhanced solubility. It also increase rate of dissolution, bioavailability and stability of the encapsulated CPH drug more effectively than conventional dosage forms. In conclusion, the results of this study indicate the potential use of the developed S-SNEDDS formulation for effective delivery of the active ingredient.

**Keywords:** *Cariprazine, SNEDDS, D-Optimal Mixture Design, Bioavailability, Cell line study*
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## 1. INTRODUCTION

CPH, atypical antipsychotic used in the treatment of schizophrenia and manic or mixed episodes of bipolar disorder. The absolute oral bioavailability was 52% in rats. Absolute bioavailability in human is unknown. CPH is extensively metabolized by hydroxylation and demethylation. It is primarily metabolized by CYP3A4.[1–3] To overcome the challenges of CPH's low solubility, slow

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dissolution rate, and poor bioavailability, there was a need for novel drug delivery dosage forms.

Lipid-based delivery systems, such as SNEDDS are recognized for their ability to enhance the solubility and absorption of lipophilic drugs by increasing the surface area and decreasing the size of the oil droplets. This results in easily digestible droplets and can be incorporated into mixed micelles capable of traversing the intestinal lumen. They are uniform liquid mixtures comprising the drug, natural or synthetic oils, surfactants, and co-surfactants, possess the distinctive capability to spontaneously generate fine oil-in-water (O/W) nano-emulsions with a size of around 200 nm or smaller, when mixed with water. This occurs under gentle agitation conditions that mimic the natural movements of the gastrointestinal tract, enhancing the drug's absorption in the body.[4–6]

SNEDDS show a significant increase in dissolution rate, solubility and permeability. It can significantly reduce p-glycoprotein efflux, first-pass effect through the liver by absorption through lymphatic circulation, and intra-individual and inter-individual variability depending on the conditions in the gastrointestinal tract. SNEDDS are thermodynamically as well as kinetically stable transparent or translucent system which was stabilized by addition of surfactants and co-surfactants. Moreover, SNEDDS has been shown to enhance transcellular permeability by improving the lipid fluidity of enterocyte membranes and inhibiting efflux pumps, which ultimately results in better oral bioavailability.[7-9]

SNEDDS have distinct advantages over conventional microemulsions and nanoemulsions, including long-term stability, better patient compliance, palatability, reduction in dosage requirements, ease of formulation, and scalability in production. Other beneficial properties of SNEDDS that contribute to enhanced oral bioavailability include minimizing cytochrome P450 metabolism in gut enterocytes, promoting lymphatic transport via Peyer's patches, and offering protection against first-pass metabolism.

There are two main types of SNEDDS: liquid SNEDDS (L-SNEDDS) and solid SNEDDS (S-SNEDDS). SNEDDS can be solidified through processes such as spray drying, melt granulation, or adsorption onto inert solid carriers like microcrystalline cellulose, Syloid XDP, Neusilin US2, Fujicalin and Aeroperl 200.

L-SNEDDS have many advantages; however, the observation of general stability problems in lipid-based systems, leakage from the oily system during packaging and transportation, the possibility of irritation in the gastrointestinal system of the high amount of surfactant used in these systems and chances of precipitation of drugs. S-SNEDDS offer a different approach to solving the issues associated with L-SNEDDS. They combine the benefits of solid dosage forms, such as cost-effectiveness, ease of control during production, high stability, better patient compliance, with improved solubility and bioavailability. Moreover, S-SNEDDS are generally preferred over L-SNEDDS due to their ease of transportation and stability.[10-12]

In this study, our goal was to develop SNEDDS for the efficient transport of CPH to the lymphatic system and avoiding its first pass metabolism. Based on their solubilising capacity and self-emulsification efficiency, we selected a combination of components, including suitable oils, surfactants, and co-surfactants, for the preparation of L-SNEDDS. We also used polymeric carriers as precipitation inhibitors to enhance the solid-state stability of L-SNEDDS by converting them into S-SNEDDS.

Currently, a wide array of statistical experimental designs has been instrumental in developing more efficient experimentation frameworks that require fewer trials while also enabling the detailed analysis of various factor significances. Within the spectrum of these statistical tools for optimization, the D-optimal mixture design stands out, especially for its application in enhancing SNEDDS (Self-Nanoemulsifying Drug Delivery Systems). This specific design approach focuses on minimizing the variance in coefficient estimates and is adept at selecting the optimal subset of variables based on predetermined criteria. This is done while considering the entirety of the SNEDDS system as a complete 100%, a consideration often overlooked by other designs. The application of the D-optimal mixture design in optimizing an SNEDDS formula containing CPH (Favipiravir) demonstrates its utility in analyzing both the primary and interactive impacts of independent variables on the response of the SNEDDS formulation. Thus, the primary goal of this research was to significantly improve the dissolution and bioavailability of CPH through the strategic formulation of SNEDDS.[13-17]

## 2. MATERIALS AND METHODS

### 2.1 Materials

CPH was generously gifted by Zydus Healthcare Pvt. Ltd., Ahmedabad, India. Capmul MCM C8, Captex 355 and other oils used in study were gifted from IMCD India Pvt. Ltd, Mumbai. Kolliphor EL, Kolliphor HS15, Tween 80, PEG 300 and other surfactants as well as co-surfactants were procured from BASF India Ltd., Mumbai. Labrasol and Transcutol HP were received as gratis sample from Gattefosse, Mumbai, India. Syloid XDP was obtained from Grace Pharmaceuticals Ltd, Mumbai, India. All other ingredients and double distilled water used for present study were of analytical grade.

### 2.2 Equilibrium Solubility Study

SNEDDS consist of oil, surfactants, co-surfactants, and the active medicament. These systems should appear as a clear, single-phase liquid at room temperature and be capable of completely dissolving the drug when mixed with water. It's crucial that the drug can dissolve well in both the oil and surfactants to prevent the drug from precipitating out of the solution when ingested orally. Various materials were tested for their ability to dissolve CPH to its saturation point. These materials include different oils (such as Castor oil, Sesame oil, Sunflower oil, Capmul GMO 50, Capmul MCM C8, Captex 200, and

Crodamol EO), surfactants (Kolliphor EL, Kolliphor HS15, Labrasol, Tween 20, Tween 80), and co-surfactants (PEG 300, PEG 400, Transcutol HP, Acrysol K140, Acrysol K150).[18-21]

To achieve this, an excess amount of the drug was added to 1 mL of each chosen vehicle within a 2 mL Eppendorf tube. The mixtures were then vortexed for 10 min to aid in dissolving, followed by 24 h on an orbital shaker at room temperature to reach equilibrium. Subsequently, the mixtures were centrifuged using a microcentrifuge at 5000 rpm for 10 min, and the clear supernatant was obtained. The drug concentration in the resulting solution was determined after dilution with methanol, and the absorption was measured at 256 nm using a UV Spectrophotometer 1800 [22]. All experiments were carried out in triplicate.

**2.3 Screening of Surfactants and Co-surfactant for Emulsification Efficiency**

Surfactants and co-surfactants were evaluated for their capability to emulsify in a chosen oil phase using a classification method for self-emulsification efficiency, as outlined in previous research. In a concise procedure, 1 ml each of surfactant and co-surfactant was mixed with 1 ml of the specified oil. These mixtures were then carefully poured into a beaker containing 200 mL of distilled water, creating a uniform nanoemulsion that showed no cloudiness or separation. The emulsions were left to settle for 2 hours, after which their light transmittance was measured at 650 nm (using a UV–Visible Double Beam Spectrophotometer) with distilled water serving as the reference. The transmittance percentages of each emulsion were determined in three repeats, and the mean values ± SD were computed. The surfactant that resulted in a transparent emulsion with the highest transmittance percentage was chosen for further use.[23, 24]

**2.4 Construction of Ternary Phase Diagram**

A ternary phase diagram is utilized to ascertain the phase behavior relationships across various combinations of oil, surfactant, and co-surfactant. The region indicative of self-nanoemulsification was pinpointed utilizing a trial iteration of the Chemix software (version 11.0). To devise the ternary phase diagram and pinpoint the self-nanoemulsification zone, a total of twenty-one experimental formulations were crafted. Diverse blends incorporating varying concentrations of surfactant, co-surfactant, and oil were formulated. Ternary diagrams representing surfactant, co-surfactant, and oil ratios were created. The proportions of oil, surfactant, and co-surfactant were adjusted from 10% to 40% (w/v), 20% to 70% (w/v), and 20% to 70% (w/v) respectively. Every composition was evaluated for its self-nanoemulsification capability by diluting each mixture 200 times with distilled water. Subsequently, the percentage of light transmission (PT) through these samples was gauged with a UV-Visible Spectrophotometer (Shimadzu Corporation 1800, Japan) at a wavelength of 650 nm. Dispersions that achieved a grade A system and exhibited more than 90% PT were deemed satisfactory.[25]

**2.5 Formulation of SNEDDS using Mixture Design**

A D-Optimal mixture design strategy was utilised to explore and fine-tune the main effects and interactions of independent variables on the outcomes within the SNEDDS formulation. Sixteen experimental configurations involving three independent variables were constructed with the help of Design Expert Software version 13 (Stat Ease, Inc, Minneapolis, USA). The chosen formulation components included the oil quantity (X1), surfactant (X2), and co-surfactant (X3). Meanwhile, the outcomes measured were the time it took for self-emulsification (Y1), the percentage of transmittance (Y2), and the average size of the globules (Y3). A factor was deemed significant when its P value was below 0.05. Multiple Linear Regression Analysis (MLRA) was applied to identify the formulation variable combination that was most efficacious. The best batch was pinpointed through the use of a desirability function.[26, 27] Details regarding the independent and dependent variables, including the actual and coded values, as well as the desirability criteria for the response variables, are outlined in Table 1.

A fixed amount of CPH (1.5 mg) was thoroughly mixed with the oil, surfactant, and co-surfactant with continuous agitation until a uniform blend was achieved. This resulting L-SNEDDS was stored at ambient temperature in airtight clear glass bottles for future analysis. Any changes in cloudiness or phase separation within the formulations were carefully documented.

**Table-1:** Factors and levels with coded and actual value for CPH SNEDDS

| **Independent Factors** | **Coded Value** | | **Actual value (%)** | |
|---|---|---|---|---|
| | Low | High | Low | High |
| **$X_1$= Amount of oil** | 0 | 1 | 10 | 40 |
| **$X_2$ = Amount of surfactant** | 0 | 1 | 20 | 50 |
| **$X_3$ = Amount of co-surfactant** | 0 | 1 | 20 | 60 |
| **Dependent variables** | | | | |
| **$Y_1$= self-emulsification time in seconds (<60sec)** | | | | |
| **$Y_2$= percentage transmittance (>90%)** | | | | |
| **$Y_3$ = Mean globule size in nm (<200nm)** | | | | |

**2.6 Optimization of SNEDDS using Mixture Design**

The chosen formulations, which incorporated varying ratios of lipid components and the drug, were assessed based on factors like the time it takes for self-emulsification, average size of the globules, and the percentage of light transmittance. These observations were documented, and multiple linear regression analysis

(MLRA) was utilized to identify the optimal batch through the use of a desirability function.

### *2.6.1 Self Emulsification Time*

This research focuses on verifying the consistency and stability of a formulation following its ingestion. A dispersibility assessment was carried out to evaluate the emulsification efficiency (emulsification speed) of certain chosen formulations included in the study design. For this purpose, a 1 mL sample from each selected formulation was dispersed into 200 mL of 0.1 N hydrochloric acid solution (mimicking gastric fluid) maintained at a temperature of 37 ± 0.5°C, with stirring at 50 rpm. The duration it took for each sample to form a nanoemulsion was recorded, and visual examinations were conducted. A grading system was utilized to determine the most appropriate formulation.[28]

### *2.6.2 Measurement of Mean Globule Size*

The critical characteristics that contribute significantly to the functionality and effectiveness of nano-based drug delivery systems include globule size, the polydispersive index, and the zeta potential of the chosen formulations. These were simultaneously assessed using a Photon Correlation Spectrometer (Zetasizer Nano ZS; Malvern Instruments, Malvern, UK). To test the stability of the SNEDDS, it was diluted 100-fold with distilled water and lightly stirred to ensure even dispersion. Subsequently, the samples, once prepared, were placed in a temperature-controlled chamber. Here, the amount of scattered light was observed at an angle of 90 degrees and a temperature of 25°C, immediately after the samples were placed in a cuvette. The reported values for size and potential are averages derived from a minimum of three separate experiments.[29]

### *2.6.3 Percentage Transmittance*

The stability of the nanoemulsion SNEDDS formulation was evaluated by measuring its percentage transmittance. During the preparation of SNEDDS for oral administration, there is a possibility that the medicine might precipitate after being diluted in the gut's lumen, hence the measurement of percentage transmittance. The chosen formula was mixed with 100 mL of distilled water and the transparency was observed at a maximum of 650 nm utilizing a UV spectrophotometer 1800. A percent transmittance greater than 90% indicates that the formulation meets the criteria for clarity.[30]

### *2.6.4 Statistical Analysis using D-Optimal Mixture Design*

The relationship between the components of the mixture and the response variables was established through a polynomial equation and analyzed statistically using the trial version of Design-Expert 13.0 software from State Ease Inc., Minneapolis, USA. The coefficients' values demonstrate the impact of these variables on the outcome. The polynomial equations include coefficients for the intercept and the interaction term, where a positive coefficient suggests a synergistic effect, whereas a negative one indicates an antagonistic impact on the outcome[31, 32]. By applying Multiple Linear Regression Analysis (MLRA) to connect the dependent and independent variables, the components of the mixture were fine-tuned for optimal responses.

In order to assess the reliability of the equations that describe the influence of the formulation factors on the response variables, two additional checkpoint experiments for CPH SNEDDS (CB1 and CB2) were conducted. The check point batches for CPH loaded SNEDDS were formulated as per the composition shown in Table 2.

**Table-2:** Formulation of checkpoint batches

| Check Point Batches | Coded Value | | | Actual Value (%) | | |
|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | $X_1$ | $X_2$ | $X_3$ |
| **CB1** | 0 | 0.3 | 0.7 | 35 | 35 | 30 |
| **CB2** | 0.3 | 0.6 | 0.1 | 15 | 45 | 40 |

### *2.6.5 Optimization of SNEDDS using Desirability Function*

Three essential elements are required to achieve optimization: a boundary value (either upper or lower), a specified target value, and an input indicating whether each outcome should be maximized or minimized. When optimization involves maximization, a lower bound value is necessary. Conversely, if a response variable is to be minimized, an upper bound value is needed. Y1 was designated for minimization since a shorter time for self-emulsification of the SNEDDS formulation to create nanoemulsion in a simulated gastric environment is preferred. Y2 was maximized, with upper and lower limits established at 100% and 90%, respectively as transparency directly reflect the size of globules. Y3 was assigned to be minimized, as a smaller particle size of the SNEDDS formulation enhances drug absorption in the gastrointestinal tract. Research on SNEDDS and nanoemulsion indicates that an ideal globule diameter should be less than 200 nm. Formulation variables in the design should be in range for optimization. The independent and dependent variables with set goal are shown in Table 3.

**Table-3:** Variables with goal as per desirability function

| Variables | Goal |
|---|---|
| X1:OIL | in range |
| X2:SURFACTANT | in range |
| X3: CO-SURFACTANT | in range |
| Y1:Emulsification time | < 60 sec |
| Y2 :%Transmittance | >90% |
| Y3 : Mean Globule size | <200 nm |

## 2.7 Methods of Characterization of Optimized L-SNEDDS

### *2.7.1 Robustness to dilution*

The capability of the SNEDDS formulation to maintain stability without undergoing phase separation or precipitation of the drug across a variety of physiological pH levels is crucial for its application in delivering medications. The optimized formulation containing CPH in SNEDDS was extended 250-fold with different solutions: distilled water, 0.1N HCl, and phosphate buffer at pH 6.8 and pH 7.4. The nanoemulsion that resulted remained clear and transparent without showing any signs of phase separation after 24 hours, indicating that the optimized SNEDDS batch was infinitely stable in aqueous dilution. Furthermore, it was observed that neither the composition nor the pH of the aqueous phase impacted the characteristics of the resulting nanoemulsions.[33]

### *2.7.2 Transmission Electron Microscopy*

Investigation into the morphology and structure of nanoemulsions was conducted through transmission electron microscopy (TEM), focusing on those prepared via specific self-emulsifying formulations. For TEM analysis, self-nano emulsifying drug delivery system (SNEDDS) samples were diluted with deionized water at a 1:200 ratio. A droplet of this diluted mixture was then placed onto coated copper grids, followed by staining with 1% uranyl acetate solution. The observations were performed using a Philips XL 30 ESEM equipped with EDAX in Eindhoven, Netherlands. [34]

### *2.7.3 Measurement of Zeta potential*

The zeta potential of oil droplets in L-SNEDDS can be determined using a zeta sizer (from Malvern Instruments, UK) to measure their zeta potential. The samples were placed into transparent disposable cuvettes for the measurements. The subsequent results provided the charge of the emulsion droplets along with their zeta potential values. Typically, a nanoemulsion is deemed stable when its charge is close to ± 30 mV. [35]

### *2.7.4 Thermodynamic stability study*

The goal of a thermodynamic stability study is to assess the effects of temperature variation and phase separation on a Self-Nanoemulsifying Drug Delivery System (SNEDDS) formulation. Unlike emulsions which merely possess kinetic stability and are prone to eventual phase separation, the SNEDDS system, through in-situ solubilization, forms a nanoemulsion system intended to maintain stability without experiencing precipitation, creaming, or cracking. This stability under thermal conditions distinguishes nanoemulsions. To evaluate this stability, an optimized batch of CPH-laden SNEDDS was subjected to tests such as centrifugation, heating-cooling cycles, and freeze-thaw cycles, utilizing a stability chamber provided by Thermolab Scientific Equipments in Mumbai. The assessment of stability was conducted by observing changes in dispersibility and transmittance.[36]

### *2.7.5 Cell line Study*

The NRK (Normal Rat Kidney) cell line was grown in Dulbecco's Modified Eagle Medium (DMEM), enriched with a variety of supplements, including L-glutamine, sodium pyruvate, nonessential amino acids (NEAA), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), and sodium bicarbonate, along with 10% fetal bovine serum. HEPES is widely utilized as a biological buffer in cell culture media, due to its ability to maintain a stable pH range over extended periods protects cells from the detrimental effects of significant pH alterations in the culture environment. Cell detachment from the culture flask was facilitated using a trypsin-EDTA solution. The viability of the NRK cells was determined through a MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay, conducted on cells that had been seeded and allowed to incubate for 24 hours. The investigation incorporated three distinct samples pertaining to the CPH drug, precisely an CPH L-SNEDDS formulation (F1), a placebo (F2), and the pure drug (F3). These samples were exposed to five different drug concentrations (15, 30, 60, 120, and 240 µg/mL) for an additional 24-hour period, followed by the introduction of the MTT dye. After a two-hour incubation, DMSO was added to each well, and the 96-well plate was left to incubate overnight. Absorbance readings were then taken at a wavelength of 570 nm using an Elisa plate reader.[37]

## 2.8 Solidification of L-SNEDDS

The optimised L-SNEDDS formulation was converted into a solid, free-flowing powder by employing Neusilin US2 as the adsorbent material, as outlined in reference [38]. A method involving adsorption onto a solid medium was utilized to transition L-SNEDDS to S-SNEDDS. This process used a constant ratio technique, in which the carrier's volume was progressively increased while maintaining the SNEDDS volume constant, to obtain a powder with free-flowing characteristics. In practice, a determined volume of SNEDDS (0.5mL) was dispensed into a petri dish, followed by a gradual addition and thorough mixing of a predetermined carrier quantity using a glass rod. This blend was then placed into a vacuum oven (Cintex Industries, Mumbai) and sealed. It was exposed to a temperature of 60°C for duration of 30 min, resulting in the formation of a homogenous powder.

## 2.9 Solid State Characterization of S-SNEDDS

### *2.9.1 Differential scanning calorimetry (DSC) study*

The thermal analysis of the pure drug, solid adsorbent, and solid self-nanoemulsifying drug delivery system was conducted using Differential Scanning Calorimetry (DSC-60, Shimadzu, Japan). To do this, a sample of the pure drug, weighing 2-3 mg, was precisely measured and placed into aluminum pans that were not airtight, before being sealed. Similarly, an empty pan was sealed following the same procedure as that for the sample pan for comparison. The DSC examination covered a temperature range from 20°C to 300°C, with the temperature increasing at a rate of 10°C per min. Throughout the analysis, nitrogen gas was flowed through at a steady rate of 50 ml per minute. The TA universal

analysis software was then used to evaluate the DSC thermogram.[39]

***2.9.2 Micromeritic Properties of S-SNEDDS***

The measurement of the angle of repose for S-SNEDDS was conducted utilizing the funnel method. A specific quantity of the substance was carefully weighed and introduced into a funnel. The funnel height was adjusted so that its tip was in contact with the top of the S-SNEDDS powder heap. Subsequently, the powder could flow unobstructed from the funnel onto a level surface. The diameter of the formed powder heap was measured to calculate the angle of repose.[40]

Furthermore, the S-SNEDDS's bulk density, tapped density, compressibility index, and Hausner Ratio were determined with a tapped density instrument (Veego Instruments, Mumbai). For these determinations, 20 g of S-SNEDDS was put into a 100 mL cylinder, and the procedure was performed in accordance with the standards prescribed in the United States Pharmacopoeia (USP). Each characteristic was evaluated three times.

***2.9.3 Surface Scanning Electron Microscopy (SEM)***

Images of the Surface-Modified Self-Nano Emulsifying Drug Delivery Systems (S-SNEDDS) were captured using a scanning electron microscope (Model: ESEM TMP + EDAX; Manufacturer: Philips, Netherland) set at a 20 kV voltage to analyze the surface structure. The samples were mounted on SEM stubs and subsequently coated with a fine platinum layer.[41]

**2.9.4 *Drug Content***

The determination of CPH content in S-SNEDDS utilized the LC-MS method. To prepare the sample, S-SNEDDS was mixed into an adequate volume of methanol, followed by a 10 min sonication to facilitate the drug's extraction into the methanol solution, which was then passed through a 0.45µ membrane filter. After ensuring the filtrate was clear, it was further diluted with methanol. The clear filtrate was transferred in 1.5 mL capacity vial and injected in LC-MS (LC Make: Shimadzu Corporation, Japan Model: Prominence and MS Make: AB Sciex LLC, USA Model: QTRAP4500). The peak area was counted and value of percentage of drug content was estimated using the equation of calibration curve.[42]

**2.9.5 *In-vitro Drug Release Study***

The study to evaluate the dissolution of CPH in S-SNEDDS was conducted utilizing a type-II paddle apparatus for dissolution (Electolab India Pvt. Ltd., Mumbai) in 500 mL of acetate buffer at pH 4.5, which was kept at a constant temperature of 37°C ± 0.5°C. The apparatus's paddle rotation speed was set at 100 revolutions per minute. Withdrawals of the sample, measuring 5 mL each, were made at intervals of 5, 10, 15, and 30 min, immediately replacing them with the same amount of new medium to preserve the sink conditions. The analysis of the concentration was performed using LC-MS/MS instrument. The cumulative release of the drug from each sample was calculated, with the entire procedure being replicated three times.[43]

**2.10 Formulation of Hard Gelatin Capsules (HGC) from S-SNEDDS**

The dimension of HGC was determined by calculating the required quantity of S-SNEDDS powder and adding appropriate diluents to reach the desired volume before filling it into HGC. The filling of the capsules is accomplished through the punch method. To seal the capsules, a tamper-evident seal technique is used, which seals at the joint where the two parts of the capsule come together (snap fit seal). Whether produced on a small or large scale, some powder may stick to the exterior of the capsules. It's important to remove this residue before the capsules are packaged or dispensed to enhance their visual appeal and maintain their quality, ensuring they are tasteless when taken. For small batches, this cleaning can be done by enclosing the capsules in a cotton cloth and gently rubbing them. To give the capsules a final polish, they are rolled on butter paper that has been coated with liquid paraffin. Any remaining excess oil is then wiped away using a cloth.[44]

**2.11 Evaluation of HGC**

The prepared HGC were evaluated for uniformity of weight, disintegration time and uniformity of content test according to the method outlined in the Indian Pharmacopoeia 2022. [45-47]

**2.12 Stability Study**

An in-depth examination of the long-term stability of formulations, influenced by temperature and humidity, was conducted by the guidelines outlined by the International Conference on Harmonisation (ICH). This research was undertaken under accelerated conditions maintained at 40 ±0.5˚C and 75 ± 5% RH for duration of six months with minimum three sampling points. The stability studies should be conducted on the capsules packaged in a container closure system that is the same as or simulates the packaging proposed for storage and distribution. The study utilized hard gelatin capsules that were packed correctly in blister packing and stored within a stability chamber that meets ICH standards, supplied by Thermolab Scientific Equipments located in Mumbai. The stability assessment of these capsules involved regular visual inspections to detect any changes in physical attributes, including appearance and weight fluctuations. Furthermore, the capsules underwent testing at predetermined intervals (initiation, one month, three months, and six months) to assess disintegration time, verify content uniformity, and perform in-vitro drug release evaluations.[48]

**2.13 Bioavailability Study**

The protocol for the animal study was approved by the Institutional Animal Ethics Committee (IAEC) under the authorization number CCSEA/IAEC/ARCP/2023-24/10. Two groups of Wistar Rats, each comprising six individuals, were established: one for the pure drug and the other for CPH loaded in S-SNEDDS HGC. Before the start of the experiment, the animals were subjected to a 12 h fasting period, though they were allowed access to tap

water. Each rat received a 1ml oral dose of either the drug powder in aqueous suspension and suspension of HGCs formulation via oral gavage. While under anaesthesia, around 0.2 mL of blood was taken at predetermined intervals of 0, 0.5, 1, 2, 4, 6, 8, 10, and 24 h from either tail vein, retro-orbital plexus, or jugular vein and was then put into EDTA-prepped Eppendorf tubes. The blood samples were spun in a centrifuge at 13000 rpm for 10 min at a temperature of 4°C to separate the plasma. The plasma underwent subsequent centrifugation with methanol to eliminate plasma proteins, after which the clear supernatant was collected. This processed plasma was kept at a temperature of -20°C until further analysis was needed. For drug quantification, the supernatant was placed into LC-MS/MS vials for subsequent assessment.[49]

### 2.14 Comparative Dissolution Profile

Dissolution studies for the pure CPH powder, CPH-loaded SNEDDS HGC, and a commercial CPH tablet were performed using a USP type II dissolution tester (Inspire Model, Electrolab India Pvt. Ltd., Mumbai). The medium for dissolution was 500 mL of acetate buffer (pH 4.5), with the test running at 50 rpm and a controlled temperature of 37 ± 0.5 °C. At predefined intervals (10, 15, 20, and 30 min), 5 mL samples were taken, filtered, suitably diluted, and then measured using LC-MS/MS instrumentation.

## 3. RESULTS AND DISCUSSION

### 3.1 Equilibrium Solubility Study

The results of solubility of CPH in various oils, surfactants and co-surfactants at room temperature are shown graphically in the Fig. 1. From the results it has been revealed that Capmul MCM C8, Tween 80 and PEG 300 depicted highest solubility for CPH and it has been selected as oil, surfactant and co-surfactant respectively. Studies on the dissolution of oily compounds have found that drugs dissolve more readily in medium-chain partial glycerides than in their long-chain unsaturated counterparts. The solubility of CPH diminishes with an increase in the glycerides' chain length. In the development of SNEDDS formulations, medium-chain triglycerides (MCT) are preferred over long-chain triglycerides (LCT) due to the simpler formulation process associated with SNEDDS.

The choice of Capmul MCM C8 as the oil phase is supported by its specific composition, aligning with the criteria mentioned earlier. Capmul MCM C8 contributes to the formulation of SNEDDS by enhancing solubility, accelerating the dissolution rate, minimizing globule size, and optimizing oral delivery.[50]

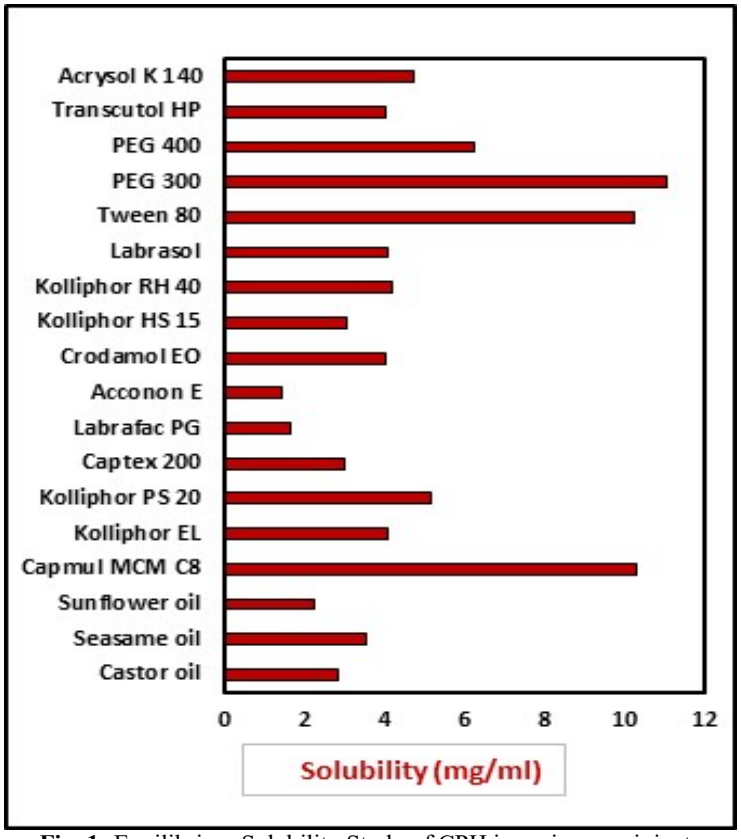


**Fig. 1:** Equilibrium Solubility Study of CPH in various excipients

### 3.2 Screening of Surfactants and Co-surfactant for Emulsification Efficiency

Although it is a major parameter in choosing the ingredients of SNEDDS, drug solubility is not the only parameter governing the choice of surfactants in the prepared systems. The surfactant's emulsifying efficiency is a much more critical factor. Therefore, the emulsifying efficiency of the selected surfactant and co-surfactant was screened regarding the selected oil. The ability of the surfactant to form an emulsion was assessed by less time needed for emulsion formation, while the stability of the formed emulsion was expressed by its percentage transmittance 2h after preparation.[51]

In this study, Tween 80 was selected as surfactant and compared for their self emulsification efficiencies with oil phase. This surfactant individually produces good emulsification with the selected oily phase. The selected co-surfactant PEG 300 an absorption enhancer, was found to be a very efficient solubilizer for CPH and showed improvement in self emulsification efficiency in combination with above mentioned surfactants.[52]

### 3.3 Construction of Ternary Phase Diagram

A series of L-SNEDDS formulations were prepared and their self-emulsifying properties were observed visually. Ternary phase diagrams were constructed in the presence of drug to identify the self nanoemulsification regions and to optimize the percentage of oil, surfactant, and co-surfactant in the L-SNEDDS formulations. Ternary phase diagrams of the systems containing Capmul MCM C8 as oil phase, Tween 80 as surfactant, and PEG 300 as co-surfactant was shown in Fig. 2.

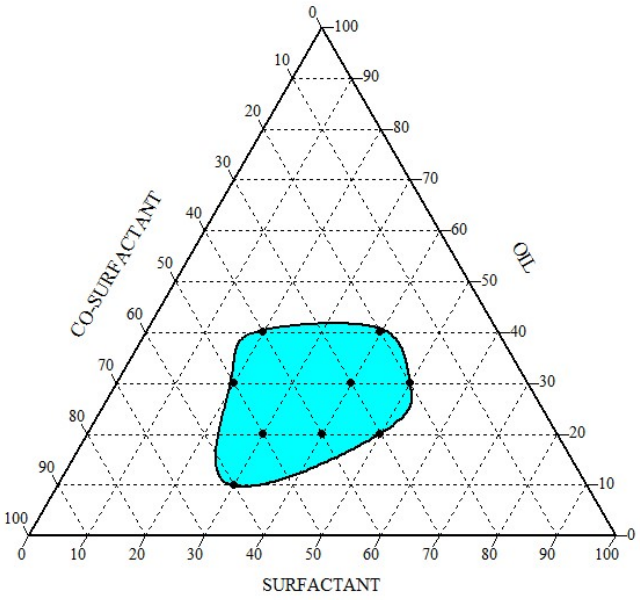


**Fig. 2:** Self nanoemulsification region in ternary phase diagram

The tendency to form an emulsion spontaneously within the self-emulsifying region increased as the percentage of surfactant in the L-SNEDDS formulation increased (Table 4). The formulation was unstable and drug was precipitated when concentration of oil increased by more than 50%. The optimum ratio of surfactant phase for self-emulsification was about 20-50% of the formula as the formulation may remain clear and there was no sign of drug precipitation.

The ratio of co-surfactant in the L-SNEDDS formulation was also a crucial factor for self-emulsification in the concentration range of 20-60%. Overall, the efficiency of self-emulsification was 'good' when the sum of the surfactant and co-surfactant ratio was above 60% of the L-SNEDDS formulation.

**Table-4:** Experimental batches for construction of ternary phase diagram

| Trial No. | Oil (%) | Surfactant (%) | Co- Surfactant (%) | Appearance | Stability/Precipitation |
|---|---|---|---|---|---|
| **EB 1** | 10.00 | 80.00 | 10.00 | Clear | UnStable (YES) |
| **EB 2** | 10.00 | 70.00 | 20.00 | Clear | UnStable (YES) |
| **EB 3** | 20.00 | 40.00 | 40.00 | Clear | Stable (NO) |
| **EB 4** | 20.00 | 50.00 | 30.00 | Clear Gel | Stable (NO) |
| **EB 5** | 30.00 | 50.00 | 20.00 | Clear Gel | Stable (NO) |

| | | | | | |
|---|---|---|---|---|---|
| **EB 6** | 30.00 | 40.00 | 30.00 | Clear Gel | Stable (NO) |
| **EB 7** | 40.00 | 40.00 | 20.00 | Clear Gel | Stable (NO) |
| **EB 8** | 30.00 | 20.00 | 50.00 | Clear | Stable (NO) |
| **EB 9** | 20.00 | 30.00 | 50.00 | Clear | Stable (NO) |
| **EB 10** | 10.00 | 30.00 | 60.00 | Clear | Stable (NO) |
| **EB 11** | 40.00 | 20.00 | 40.00 | Clear | Stable (NO) |
| **EB 12** | 50.00 | 20.00 | 30.00 | Turbid | UnStable (YES) |
| **EB 13** | 60.00 | 30.00 | 10.00 | Turbid | UnStable (YES) |
| **EB 14** | 10.00 | 10.00 | 80.00 | Clear | UnStable (YES) |
| **EB 15** | 20.00 | 10.00 | 70.00 | Clear | UnStable (YES) |
| **EB 16** | 30.00 | 10.00 | 60.00 | Clear | UnStable (YES) |
| **EB 17** | 40.00 | 10.00 | 50.00 | Turbid | UnStable (YES) |
| **EB 18** | 50.00 | 10.00 | 40.00 | Turbid | UnStable (YES) |
| **EB 19** | 60.00 | 10.00 | 30.00 | Turbid | UnStable (YES) |
| **EB 20** | 70.00 | 10.00 | 20.00 | Turbid | UnStable (YES) |
| **EB 21** | 80.00 | 10.00 | 10.00 | Turbid | UnStable (YES) |

**Formulation and Optimization of SNEDDS using Mixture Design**

SNEDDS formulations were prepared using Capmul MCM C8, Tween 80 and PEG 300 as the oil, surfactant and co-surfactant respectively. Amount of drug (1.5 mg) kept constant in all sixteen runs and SNEDDS formulations were prepared as per the method mentioned earlier in literature. The effect of three independent variables, Amount of oil (X1), Amount of Surfactant (X2) and Amount of co-surfactant (X) was studied on the responses viz; self emulsification time (Y1), percentage transmittance (Y2), and mean globule size (Y3) of developed formulation.

**Table-5:** Results of experimental mixture design batches

| Batch No | X1(%) | X2(%) | X3(%) | Y1 (s) | Y2 (%) | Y3 (nm) |
|---|---|---|---|---|---|---|
| **F1** | 31 | 40 | 29 | 56.23 ± 3.14 | 95.2 ± 0.005 | 72.74 |
| **F2** | 30 | 50 | 20 | 68.85 ± 4.56 | 90.7 ± 0.004 | 78.21 |
| **F3** | 10 | 50 | 40 | 76.57 ± 2.58 | 87.5 ± 0.012 | 56.45 |
| **F4** | 28 | 23 | 49 | 48.58 ± 5.38 | 97.3 ± 0.008 | 68.03 |
| **F5** | 25 | 35 | 40 | 39.13 ± 1.23 | 98.2 ± 0.005 | 64.31 |
| **F6** | 17 | 23 | 60 | 49.45 ± 3.74 | 97.2 ± 0.005 | 45.65 |
| **F7** | 10 | 50 | 40 | 74.23 ± 6.89 | 85.6 ± 0.065 | 65.87 |
| **F8** | 30 | 50 | 20 | 64.54 ± 5.24 | 91.8 ± 0.007 | 73.81 |
| **F9** | 40 | 30 | 30 | 38.32 ± 3.27 | 98.9 ± 0.023 | 92.6 |
| **F10** | 20 | 50 | 30 | 62.45 ± 6.35 | 92.4 ± 0.054 | 63.91 |
| **F11** | 40 | 40 | 20 | 54.92 ± 5.12 | 96.2 ± 0.007 | 98.04 |
| **F12** | 10 | 38 | 52 | 40.66 ± 4.89 | 98.2 ± 0.039 | 59.3 |
| **F13** | 40 | 20 | 40 | 22.75 ± 2.85 | 99.9 ± 0.002 | 84.31 |
| **F14** | 17 | 23 | 60 | 47.78 ± 4.28 | 97.2 ± 0.048 | 42.36 |
| **F15** | 40 | 40 | 20 | 56.35 ± 3.47 | 96.2 ± 0.006 | 102.68 |
| **F16** | 40 | 20 | 40 | 26.56 ± 2.86 | 99.8 ± 0.002 | 86.35 |

The self-emulsification time was found to be in the range of 22.75-76.57 s whereas batch F13 was showing the lowest self emulsification time (22.75 ± 2.85 s). The percentage transmittance of all 16 batches was found to be in the range of 85.6-99.9%. Formulations containing high concentration of surfactant were clear and transparent,

whereas formulation containing high concentration of oil was not transparent. Batch F13 was superior to other batches having 99.9 ± 0.002% transmittance showing that the formulation is clear and transparent. Mean globule size for all formulations were found to be in the range of 42.36 to 102.68 nm. The results of all three response variables are depicted in Table 5.

The duration of emulsification was the criterion for evaluating the emulsification effectiveness, which necessitates complete and swift dispersion upon mild stirring in water. An ideal benchmark was established as duration shorter than 2 min for assessing the emulsification efficacy. The self-emulsification process should happen with little agitation and shouldn't be dependent on a negative free energy. In all chosen formulations, the amount of time needed for self-emulsification incremented with an increase in oil content and reduced with a decrease in Smix content, and the opposite was also true.

Higher value of percentage transmittance was an indication that the finished globule size has a nanometer scale. Transmittance was measured to evaluate the stability of the optimized nanoemulsion of SNEDDS. It also gave a proposal about the features of formulation, such as the droplet's size and uniformity. It was found, which confirmed its clarity after dispersion into distilled water. Also, it confirmed that there are no chances of drug precipitation and optimized formulation had good solubilisation capacity after dispersion.

In the formulation of CPH L-SNEDDS, it was found that as the concentration of the surfactant increased and the concentration of oil decreased, there was a reduction in the size of the globules observed. It appeared that the size of the particles became smaller with lesser amounts of oil and larger with greater amounts of surfactant. This decrease in particle size within the SNEDDS could potentially result in a faster release of the drug. The diminished droplet size could be explained by the hydrophilic nature of the surfactant and the hydrophobic characteristics of the co-surfactant.

### 3.4 Statistical Analysis using D-Optimal Mixture Design

Table 6 details the MLRA data for CPH-loaded L-SNEDDS, demonstrating that the responses fit well with linear, quadratic, and unique cubic models.

**Table-6:** Multiple regression analysis data for CPH L-SNEDDS

| Co-efficient | Y1 | Y2 | Y3 |
|---|---|---|---|
| **Linear Mixture** | 2794.40 | 211.81 | 3867.66 |
| $X_1X_2$ | 35.47 | 1.08 | 92.09 |
| $X_1X_3$ | 134.75 | 9.84 | 205.33 |
| $X_2X_3$ | 41.85 | 4.49 | 42.37 |
| $X_1^2X_2X_3$ | 49.16 | 3.33 | - |
| $X_1X_2^2X_3$ | 161.63 | 11.47 | - |
| $X_1X_2X_3^2$ | 52.77 | 5.36 | - |
| **Model (Suggested)** | Cubic | Cubic | Quadratic |
| **P value** | < 0.0001 | < 0.0001 | < 0.0001 |
| **$R^2$ value** | 0.9916 | 0.9868 | 0.9961 |
| **Lack of Fit** | 9.51 | 1.28 | 1.28 |

The regression's standard error (SE) describes the average distance from the data points to the regression line. In contrast, the predicted residual error sum of squares (PRESS) evaluates the model's fit to the data, with lower values indicating better predictive capabilities. The R-squared value explains how much of the variability in a response variable is accounted for by the independent variables in the model, with a higher value suggesting a more comprehensive explanation by the model.

For the response variable Y1, the significance of the interaction terms is greater than that of the linear terms for L-SNEDDS, as depicted in Figure 3. This indicates that the interaction between oil and the combination of surfactant and co-surfactant is crucial for self-emulsification. The primary goal was to assess the combined effects of the three independent variables to determine their optimal composition. Hence the focus was on the magnitude of the linear effect parameters.

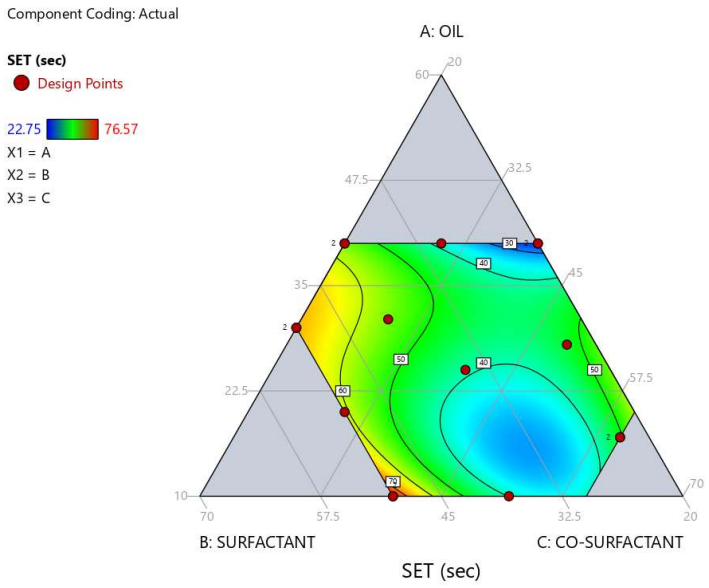


**Fig. 3:** Contour plot representing effect of independent variables on Y1

For response Y2, the co-efficient value of only linear terms is significant in the model for both formulations of SNEDDS. It may be noted that the interactive term (mixture of oil and Smix) has a less significant effect on the transparency of nanoemulsions loaded with CPH. The co-efficient value of interactive terms is not observed in the MLRA data of CPH loaded SNEDDS formulation shown in Fig. 4.

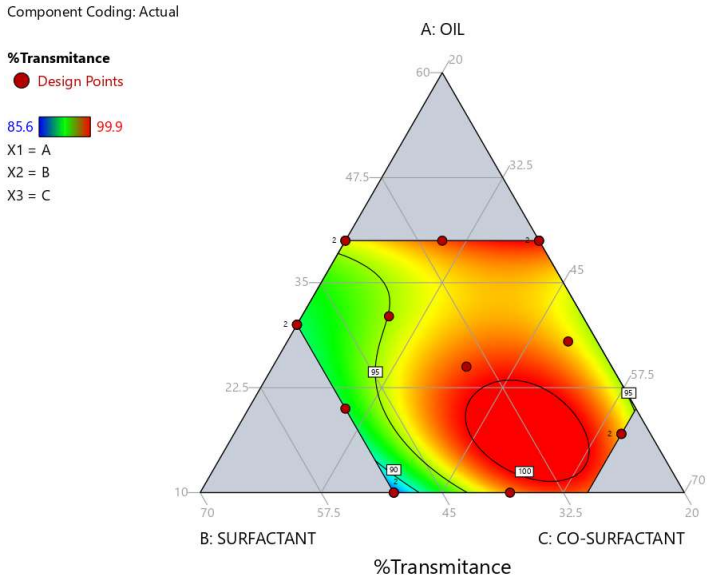


**Fig. 4:** Contour plot representing effect of independent variables on Y2

The level of change in the response variable Y3 was primarily affected by X1, as shown in Fig. 5. A positive effect of X1 (oil composition) on Y3 suggested a decrease in Y3 with a decrease in X1. Notably, the interactive term (mixture of oil surfactant and co-surfactant) has a more significant effect on decreasing the globule size. The data reveal that droplet size values are influenced by the composition of oil components and a mixture of surfactant/co-surfactants found in the D-optimal mixture design.

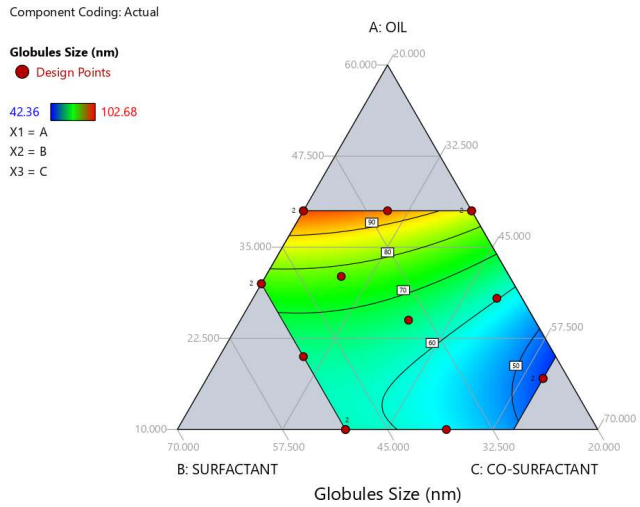


**Fig. 5:** Contour plot representing effect of independent variables on Y3

In order to assess the reliability of the equations that describe the influence of the formulation factors on the response variables, two additional checkpoint experiments for CPH L-SNEDDS were conducted. The results of check point batches are shown in Table 7.

**Table-7:** Results of checkpoint batches

| Responses | CB1 | | | | CB2 | | | |
|---|---|---|---|---|---|---|---|---|
| | **Predicted Mean** | **Observed mean** | **95% CI Low for Mean** | **95% CI High for Mean** | **Predicted Mean** | **Observed mean** | **95% CI Low for Mean** | **95% CI High for Mean** |
| Y1 (Sec) | 51.52 | 52.85 | 49.32 | 54.65 | 48.86 | 44.32 | 45.42 | 52.62 |
| Y2 (%) | 95.98 | 96.2 | 92.56 | 98.54 | 95.55 | 94.2 | 92.67 | 100.45 |
| Y3 (nm) | 83.98 | 87.45 | 79.48 | 88.12 | 60.64 | 64.31 | 58.78 | 66.95 |

It was noted that the observed mean for all three responses was found to be in the range of a 95% confidence interval, and it was also nearer to the predicted mean. Hence, the applied model was validated. The results of mixture design for all three responses show satisfactory result for all three responses.

Batch F13 containing 40% oil, 20% surfactant and 40% co-surfactant was considered as optimized formulation which shows satisfactory result as shown in Table 8 for the response variables as per the goal set for desirability criteria. The overlay plot of desirability function is shown in Fig. 6.

**Table-8:** Results of optimized batch as per desirability function

| **Variables** | **Solution for CPH SNEDDS** |
|---|---|
| X1:OIL | 40% |
| X2:SURFACTANT | 20% |
| X3: CO-SURFACTANT | 40% |
| Y1:Emulsification time | 24.92 sec |
| Y2 :%Transmittance | 99.94% |
| Y3 : Mean Globule size | 86.3 nm |
| Desirability for Batch F13 | 0.98 |

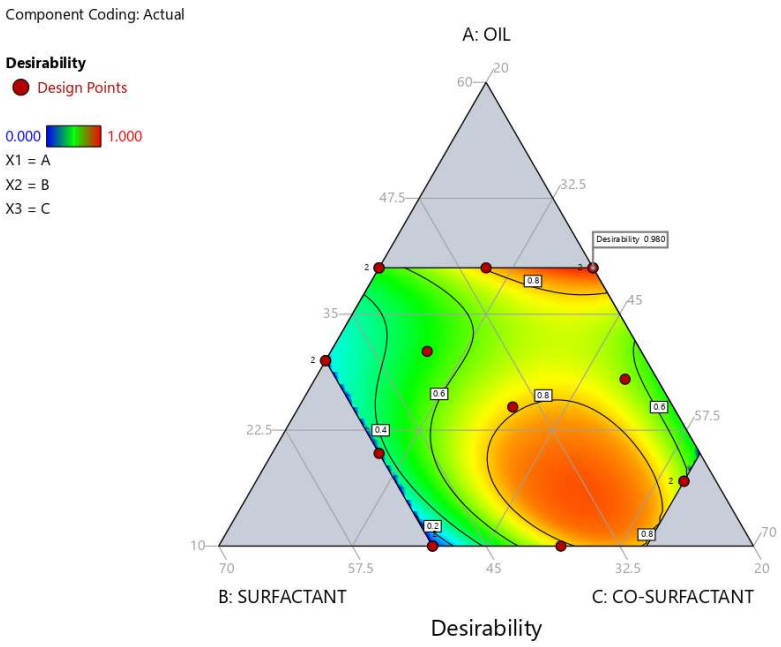


**Fig. 6:** Overlay Plot of Desirability Function

### 3.5 Characterization of Optimized L-SNEDDS

Batch F13 CPH loaded SNEDDS formulation was diluted 250 times of its weight with distilled water, 0.1N HCl, phosphate buffer (pH 6.8 and pH 7.4). The resulting nanoemulsion was found to remain transparent and clear, and showed no phase separation when kept for 24 h which conclude that optimized SNEDDS batch was stable at infinite aqueous dilution and it was qualified as robust formulation. In addition, the composition and pH of the aqueous phase was found to have no effect on the properties of formed nanoemulsions.

Studies using transmission electron microscopy (TEM) were conducted to reveal morphology and structure of the nanoemulsion produced from selected one-emulsifying phrasings. In order to observe the oily droplets, the CPH loaded SNEDDS formulation was turned in to nanoemulsion by diluting with distilled water. A representative TEM picture is shown in Fig. 7 showing different size of nanoglobules with spherical shape. It is evident that all the oil globules are of uniform size and spherical shape which clearly revealed that optimized batch produce nanoemulsion of desired globule size.

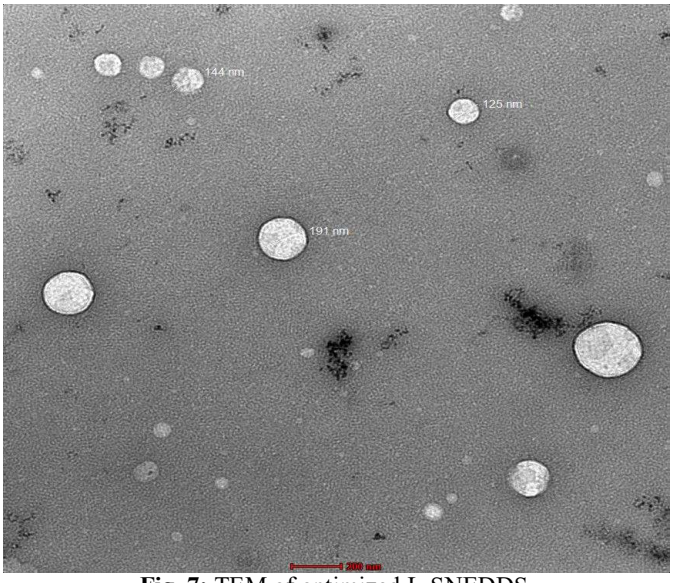


**Fig. 7:** TEM of optimized L-SNEDDS

The Zeta potential was found to be -30.9 mV; which indicate that the prepared optimized L-SNEDDS formulation was stable as shown in Fig. 8. In general, nanoemulsion is considered to be stable, if the charge is nearer to ± 30 mV. Here zeta potential of optimized batch of CPH loaded SNEDSS (F13) was met the criteria. Although, the negative value of zeta potential has indicated presence of free fatty acids which maintain and improve the stability of formulations by preventing globule from precipitation separation/coalescence.

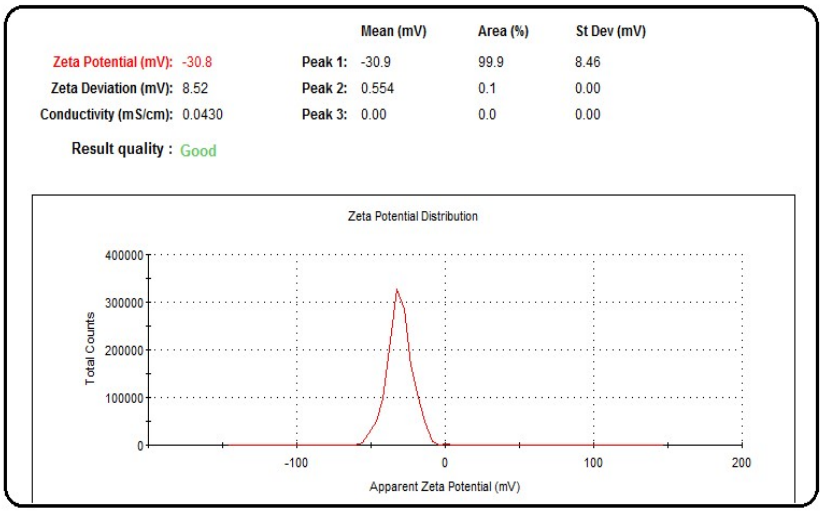


**Fig. 8:** Zeta Potential of optimized L-SNEDDS

The zeta potential significantly influences nanoemulsions' stability, making the measurement of this parameter crucial for stability assessments. When the zeta potential is high, it suggests strong electrostatic repulsion between droplets. A new type of SNEDDS results in negatively charged oil droplets when diluted with water. This leads to the droplets adhering to the intestinal mucosa, facilitating drug uptake from the mucosa. Consequently, these formulations improve oral absorption efficiency and, thereby, oral bioavailability.

The optimized (F13) formulations was subjected to different thermodynamic stability parameters by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. It was observed that formulation pass the all three tests and was found to be thermodynamically stable.

To evaluate the toxicity of surfactant and co-surfactants in the formulation of CPH SNEDDS, the MTT assay was utilized on the normal rat kidney (NRK) cell line, which was plated and incubated for 24 h. The evaluation was conducted on three samples: the CPH L-SNEDDS formulation (C1), a placebo (C2), and the pure drug (C3). It was noted that the samples containing only the pure drug exhibited lower cell viability compared to the placebo samples, which included excipients, as indicated in Fig 9, demonstrating higher cell viability. This suggests that the L-SNEDDS formulation, which was composed using capmul MCM C8, Tween 80, and PEG 300, had a safer profile for oral administration relative to the pure drug. Nano drug delivery systems are crafted to enhance the permeation of loaded drugs through biological membranes. Due to their distinctive composition, SNEDDS are favored for their superior ability to permeate biological membranes.

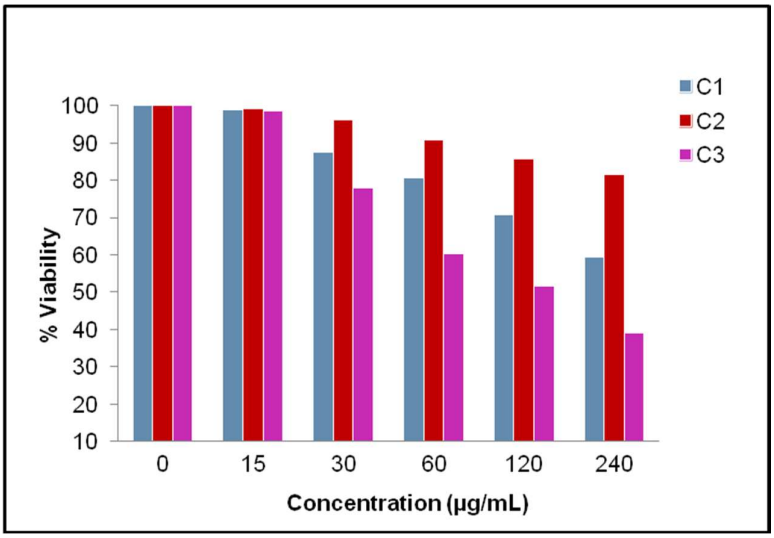


**Fig. 9:** In vitro cell line study of optimized L-SNEDDS

### 3.6 Solidification of L-SNEDDS

S-SNEDDS was prepared with Aeroperl 300 and Neusilin US2 produced good S-SNEDDS in terms of flowability and reconstitution. While S-SNEDDS prepared with MCC PH-200 and Syloid XDP had comparatively fair flowability. S-SNEDDS prepared using Fujicalin SG, was sticky in nature and difficult to handle with poor flowability. From the result of the preliminary trials as shown in Table 9 the amount of solid carrier required for solidification CPH loaded L-SNEDDS is less for Syloid XDP. It has good adsorption capacity as compared to other carrier with excellent flowability, hence it was selected as porous carrier.

**Table-9:** Adsorption capacity of solid carrier

| Sr. No. | Name | Amount of carrier* |
|---|---|---|
| **1** | Aeroperl 300 | 175.67 ± 4.041 |
| **2** | Fujicalin SG | 300.67 ± 3.055 |
| **3** | MCC PH-200 | 276.67 ± 2.887 |
| **4** | Neusilin US2 | 192.33 ± 2.517 |
| **5** | Syloid XDP | 166.67 ± 2.887 |

*Mean ± SD (n=3)

### 3.7 Solid State Characterization of S-SNEDDS

DSC thermogram of pure drug, Syloid XDP and CPH S-SNEDDS is shown in Fig 10.

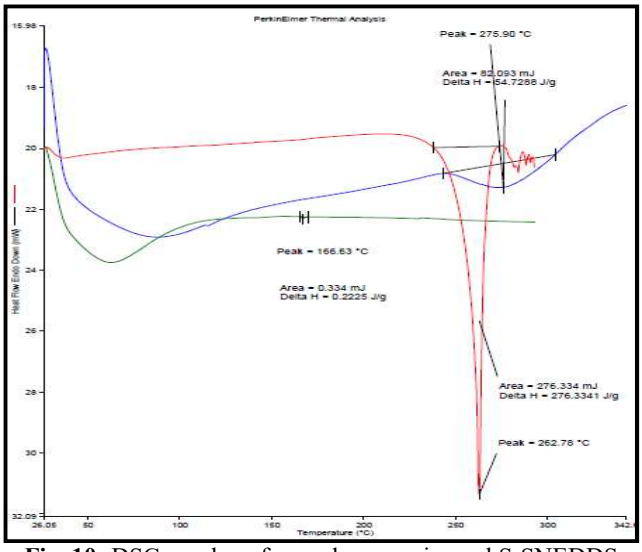


**Fig. 10:** DSC overlay of pure drug, carrier and S-SNEDDS

When pure CPH was analyzed using DSC, a narrow and sharp peak with a temperature of 262.78°C was observed, indicating the melting point of the pure compound. However, when loaded with S-SNEDDS, there was no sharp endothermic peak observed. This suggests that CPH remains in a molecularly dissolved state within the S-SNEDDS, which confirms the presence of drug in an amorphous form.

The results of flow properties of CPH S-SNEDDS are shown in Table 5. The angle of repose and all other flow property parameters were found satisfactory. The prepared S-SNEDDS has good flow characteristics.

**Table-10:** Results of flow properties of S-SNEDDS

| Parameters | Results (Mean ± SD) |
|---|---|
| Bulk density | 0.69 ± 0.033 gm/cc |
| Tapped density | 0.75 ± 0.024 gm/cc |
| Carr's Index | 8 % |
| Hausner's ratio | 1.086 |
| Angle of repose | 29.54 ° ± 1.974 |

The morphological features of S-SNEDDS were observed by scanning electron microscope. The SEM of pure Neusilin US2 and CPH S-SNEDDS are shown in Fig. 8. It showed that S-SNEDDS appeared as smooth surfaced particles, indicating that the L-SNEDDS is adsorbed or coated inside the pores of Neusilin US2 with a lesser amount of aggregation. The image showed an adsorption of drug particles on a porous solid carrier with no sign of any crystallization.

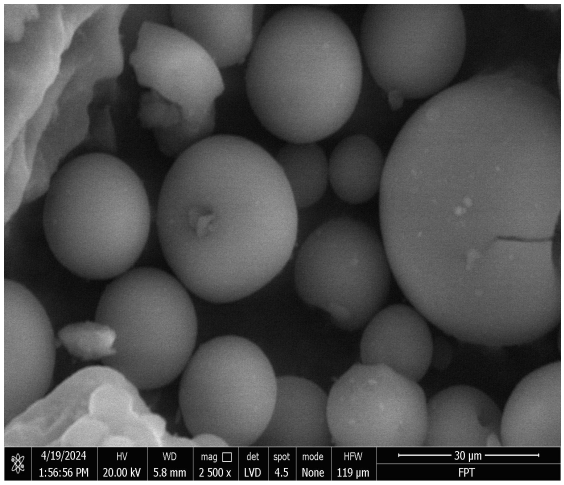


**Fig. 11:** SEM image of S-SNEDDS

The efficiency of drug loading was determined to be 98.48±0.025%, as calculated using the calibration curve method for CPH integrated into S-SNEDDS (combined with Syloid XDP). This determination involved triplicate measurements across three distinct samples.

Figure 12 presents the in-vitro dissolution profile of the S-SNEDDS containing CPH, showing a quick dissolution rate with 98.58 ± 0.895 cumulative drug release (CDR) within 30 min. The rate at which the drug is released from the SNEDDS is mainly dependent on its solubility, as the droplet size appeared to have no significant impact on CPH release. This suggests that the SNEDDS formulation plays a critical role in boosting the dissolution rate by enhancing the solubility of the medication. This improvement in dissolution with the SNEDDS could stem from the drug being in a solubilized form within this specific formulation. Upon interaction with the dissolution medium, small droplets form that dissolve rapidly.

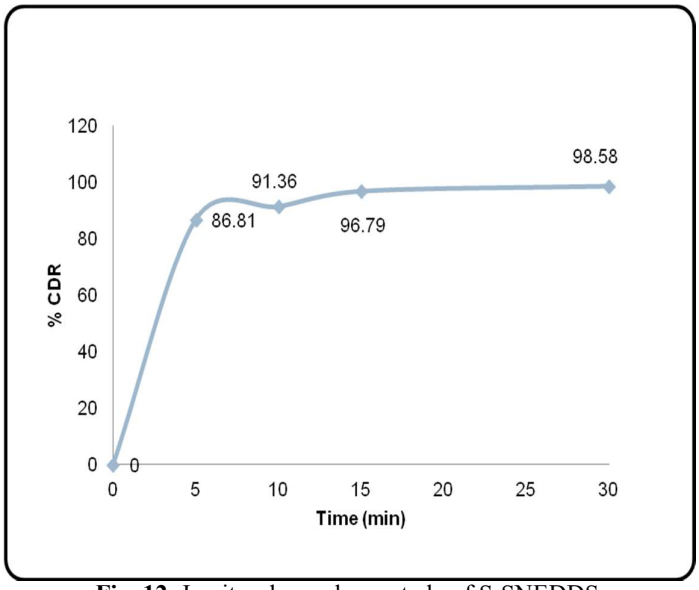


**Fig. 12:** In vitro drug release study of S-SNEDDS

### 3.8 Formulation and Evaluation of HGC

The evaluation parameters are shown in Table 11. Capsules were passed from uniformity of weight test as per the acceptance criteria specified in IP 2022. The disintegration time of CPH loaded S-SNEDDS capsule was found to be in the range of 21.43 to 27.23 min. The average disintegration time of HGC was found to be 24.82 which is less than limit specified in Indian Pharmacopoeia. The capsules were complies with the uniformity of content because all ten units falls within the specified limit 85-115%.

**Table 11:** Evaluation parameters of HGC

| Evaluation Parameters | Results | Interference |
|---|---|---|
| Uniformity of weight | 0.978 – 1.021% SD | PASS |
| Disintegration time | 5.74 min | PASS |
| Uniformity of content | 92.52 – 103.45 | PASS |

The results of the stability study are depicted in Table 12. Data shows that there was no significant change in the physical appearance. Results of disintegration time, content uniformity and in-vitro drug release shows no significant change in the parameter so it was concluded that the prepared HGC formulation was stable at accelerated storage condition as per ICH guidelines.

**Table 12:** Results of Stability Study

| Dosage Form | Time | Stability Parameters | | |
|---|---|---|---|---|
| | | Disintegration time (min) | Uniformity of Content % | % CDR |
| CPH loaded S-SNEDDS HGC | 0 day | 5.74 | 90.87-105.78% | 98.56 ± 0.852% |
| | 1 month | 6.42 | 90.62-101.84% | 96.45 ± 1.286% |
| | 3 months | 5.42 | 89.48-100.64% | 97.72 ± 2.624% |
| | 6 months | 5.56 | 86.43-102.78% | 96.12 ± 0.985% |

In vivo pharmacokinetic behaviours of CPH S-SMEDDS capsules and pure drug were studied in rat. Mean plasma concentration was plotted as a function of time as shown in Fig 13.

The noncompartment model is used to evaluate pharmacokinetic parameters of CPH absorption which are summarized in Table 13. The linear trapezoidal rule is used to calculate the area under curve (AUC0→t). Plasma concentration $C_{max}$ and $AUC_{0\rightarrow t}$ are significantly increased for formulation than those for the pure drug suspension.

Tmax is decreased for formulation and it was 60 min for formulation and 2 h for pure drug suspension. Relative bioavailability is 162.5% which indicate that there was 1.6 fold increases in the bioavailability in formulation. The results of AUC0→∞ were compared using t test, and it was found that it is highly significant ($p < 0.05$) when formulation and drug suspension were compared.

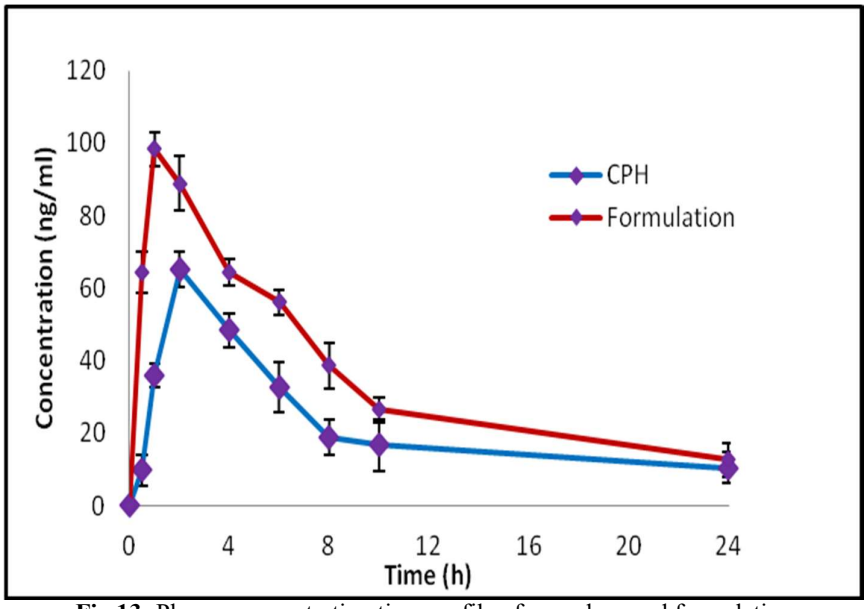


**Fig 13:** Plasma concentration time profile of pure drug and formulation

**Table-13:** Pharmacokinetic parameters of non compartmental model

| Parameters | Pure drug | Formulation |
|---|---|---|
| $C_{max}$(ng/ml) | 65.25 | 96.34 |
| Half life (h) | 19.35 | 7.69 |
| $T_{max}$ (h) | 2 | 1 |
| AUC0→t (ng h/mL) | 538.12 | 874.03 |
| AUC0→∞ (ng h/mL) | 829.045 | 1013.68 |
| MRT0→∞ (h) | 23.513 | 11.15 |
| Relative bioavailability (%) | - | 162.5 |

The improved bioavailability of S-SNEDDS could be attributed to its transportation through the transcellular pathway in the lymphatic system. Additionally, MCT oils have been identified to enhance lipoprotein formation and subsequent absorption into the lymphatic system. The presence of a single layer of intestinal epithelial cells presents the most significant obstacle to the absorption/diffusion of drugs. The elevated levels of surfactants in S-SNEDDS are thought to boost permeability by disrupting the cell membrane structure.

It's essential to recognize that the most effective surfactants for enhancing absorption possess hydrophilic and lipophilic properties that achieve a balance, often at intermediate HLB values. Surfactants have also been shown to temporarily open tight junctions, likely by interacting with the lipid bilayers' polar head groups, thus altering hydrogen bonding and ionic interactions. From studies in vitro and in vivo, it is plausible to suggest that an augmented release pattern of CPH from S-SNEDDS could enhance the drug's bioavailability.

### 3.9 Comparative Dissolution Profile

*In-vitro* drug release of formulation was compare with the pure drug and marketed tablet formulation i.e. Cariprec Capsule (1.5 mg). Pure drug shows only 32.78% drug release after 30 min, Marketed product shows 45.86% whereas HGC shows 98% drug release within 30 min as shown in Fig. 14. This data clearly indicate that by formulating S-SMEDDS formulation of drug, solubility and thus dissolution profile of was increased as compared to pure drug powder and marketed tablet.

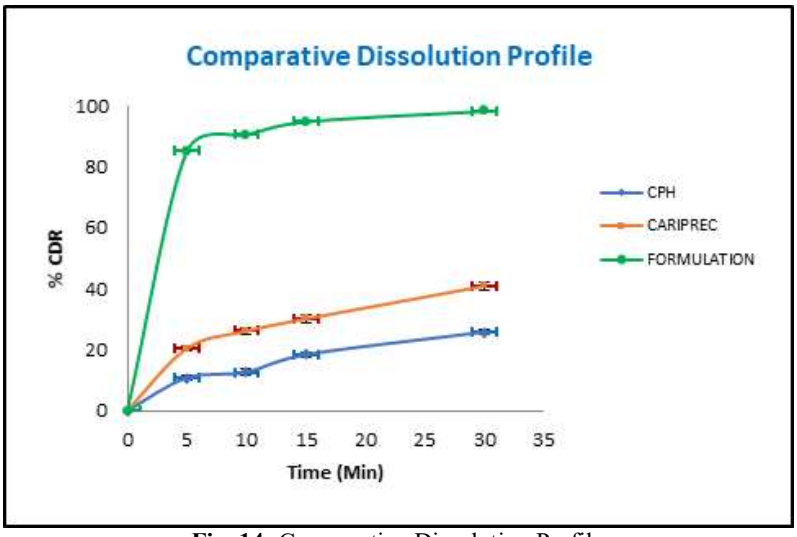


**Fig. 14:** Comparative Dissolution Profile

## 4. CONCLUSION

This study has successfully created SNEDDS utilizing medium-chain triglycerides (MCT) to enhance the bioavailability of the poorly soluble drug CPH. The L-SNEDDS demonstrated a significant reduction in mean globule size and exhibited strong thermodynamic stability. By using Syloid XDP as an adsorbent, L-SNEDDS was transformed into S-SNEDDS, resulting in a product with favorable flow characteristics suitable HGC production. These capsules maintained stability in both appearance and drug content over a six-month period. Bioavailability assessments revealed a significant increase (1.6 times) in relative bioavailability compared to the drug in its pure form.

In conclusion, method aims to enhance the solubility of poorly water-soluble drugs like CPH. The Design of Experiments (DoE) methodology facilitated formulation scientists in efficiently identifying ingredient interactions and reducing the number of experiments needed for optimization. Hence, DoE is a key tool for recognizing and managing variables vital for scaling production while preserving the pharmaceutical effectiveness demonstrated in laboratory settings. The S-SNEDDS formulations effectively retained the rapid and complete drug release characteristics of the original L-SNEDDS, even after prolonged storage. These findings suggest that the S-SNEDDS approach is promising, especially when combining L-SNEDDS with a suitable polymeric carrier. This formulation strategy presents an innovative solution for developing solid oral dosage forms for drug candidates with low solubility and has potential for creating stable solid formulations that can enhance oral bioavailability. Future research is likely to further investigate this formulation strategy.

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### Conflict of Interest

We assert that there are no conflicts of interest, either personal or institutional, that have compromised the integrity of the work reported in this manuscript.

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### Author Contribution

All authors contributed to the conception and design of the study. Suthar RM wrote the first draft of the manuscript. Solanki AB revised and reviewed the manuscript. Data collection, analysis, and interpretation were performed by Prajapati PN. Palva RS paraphrased the final manuscript and checked its plagiarism. Shah UV has performed a cell viability study for this research. A comparative dissolution profile was conducted by Detholia KK. All authors read and agreed to the final version of the manuscript for publication.

### Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Ethical approval and consent to participate

The bioavailability studies were carried out in accordance with the protocols of the Institutional Animal Ethics Committee (IAEC) under the authorization number CCSEA/IAEC/ARCP/2023-24/10.

**Competing interests**

The authors declare that none of the findings described in this study could have been driven by any prior competing financial interests or personal connections.

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