https://doi.org/10.33472/AFJBS.6.10.2024.3729-3747

African Journal of Biological Sciences

Development Of Curcumin Self-Nanoemulsifying Drug Delivery Systems For Combatting Mucositis

Mr. Mahesh Bharati[1], Dr. Swaroop Lahoti[2*]

[1]Dept of Pharmaceutics, YB Chavan College of Pharmacy, Aurangabad-431001, India
[2*]Professor Y. B. Chavan College of Pharmacy Dr. Rafiq Zakaria Campus, Dr Rafiq Zakaria Marg, Rauza Baug, N 2, CIDCO, Aurangabad, Maharashtra 431003, India Mobile number: +91 9823371119
Email ID: pharmalahoti@gmail.com
***Corresponding Author:** Dr. Swaroop Lahoti
*Professor Y. B. Chavan College of Pharmacy Dr. Rafiq Zakaria Campus, Dr Rafiq Zakaria Marg, Rauza Baug, N 2, CIDCO, Aurangabad, Maharashtra 431003, India Mobile number: +91 9823371119
Email ID: pharmalahoti@gmail.com

Article History
Volume 6,Issue 10, 2024
Received:20 Apr 2024
Accepted : 11 May 2024
doi:10.33472/AFJBS.6.10.2024.3729-3747

Abstract
This study aimed to optimize a self-nanoemulsifying drug delivery system (SNEDDS) for curcumin (CUR) to tackle its poor solubility and bioavailability. Preliminary screening identified optimal components based on solubility, emulsifying efficiency, and pseudo-ternary phase diagram construction. Optimization was performed using Design Expert® software, considering droplet size, Polydispersity Index (PDI), and transmittance as responses. The formulation, comprising Caprylol 90 and Capmul MCM C-8 oils, Cremophor EL, Tween 80 surfactants, and Gelucire 48/16 co-surfactant, demonstrated a globule size of 181.3 ± 6.5 nm, PDI of 0.27, and negative zeta potential (-11.7 mV), ensuring stability and uniformity. Morphological analysis confirmed the presence of uniform, spherical oil globules with a high drug content of 98.2 ± 1.64%. In vitro studies revealed a rapid drug release, surpassing 85% within 2 hours, notably higher than plain CUR. Pharmacokinetic assessment in vivo indicated enhanced Cmax, AUC, t1/2, and MRT for CUR-loaded SNEDDS, suggesting improved bioavailability and extended systemic exposure. These results highlight the potential of the optimized SNEDDS formulation in ameliorating clinical outcomes, particularly in conditions like mucositis, where enhanced drug delivery is critical.
Keywords: Self-nanoemulsifying Drug Delivery System; Curcumin; Nanoemulsions; Pharmacokinetics; Solubility; Bioavailability

Introduction

Oral mucositis, a distressing condition characterized by inflammation and ulceration of the oral mucosa, is a common and debilitating side effect of cancer therapy, particularly chemotherapy and radiotherapy (Pulito et al., 2020). Approximately 30-40% of individuals undergoing chemotherapy treatment encounter the debilitating condition of mucositis. This prevalence escalates to a striking range of 60-85% among patients undergoing hematopoietic stem cell transplantation. Notably, head and neck cancer patients subjected to a combined regimen of radiotherapy and chemotherapy face an even higher incidence, reaching almost 90%. The heightened vulnerability of these specific patient groups underscores the urgent need for targeted interventions to alleviate the significant burden

imposed by mucositis in the context of cancer treatment (Villa & Sonis, 2016). It significantly impacts the quality of life of affected individuals and may lead to treatment interruptions, dose reductions, and increased healthcare costs (Shetty et al., 2022). The pursuit of effective therapeutic strategies to ameliorate oral mucositis has spurred considerable research interest, and one promising avenue involves harnessing the potential of curcumin (1, 7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) (Ramezani et al., 2023). Curcumin, the principal polyphenolic compound extracted from Curcuma longa, exhibits a spectrum of pharmacological properties, including anti-inflammatory (Ramezani et al., 2023), antioxidant (Jakubczyk et al., 2020), and anticancer effects (Gholipour et al., 2023). Its multifaceted attributes make it an attractive candidate for mitigating the inflammatory processes associated with oral mucositis. However, despite the therapeutic promise, curcumin's clinical efficacy has been hampered by its poor aqueous solubility and low bioavailability (Górnicka et al., 2023). It is reported that the solubility of curcumin in aqueous solution (pH 5.0) was only 11 ng/ml (Tønnesen et al., 2002). The conventional administration of curcumin faces challenges owing to its limited water solubility, which results in suboptimal absorption and bioavailability (Hegde et al., 2023). Previous attempts at enhancing curcumin's oral bioavailability have included the use of various formulations such as liposomes (De Leo et al., 2018), nanoparticles (Chopra et al., 2021), and cyclodextrins (Jiang et al., 2022). While these strategies have shown some improvement, they often present formulation complexities and scale-up challenges. In response to the shortcomings of conventional delivery systems, the focus has shifted towards innovative nanotechnology-based approaches, particularly self-nanoemulsifying drug delivery systems (SNEDDS). SNEDDS offer a versatile and efficient means of delivering hydrophobic drugs by forming nano-sized emulsions in the presence of gastrointestinal fluids (Abushal et al., 2022). This enables enhanced solubility, stability, and ultimately, improved bioavailability of lipophilic compounds (Baloch et al., 2019). The application of SNEDDS to curcumin delivery holds promise in addressing the bioavailability challenges associated with its oral administration. Studies have shown that SNEDDS formulations can significantly improve the dissolution rate and absorption of poorly water-soluble drugs, resulting in enhanced therapeutic outcomes (Krstić et al., 2018). The nanoscale nature of the emulsions formed by SNEDDS facilitates efficient drug transport across biological barriers, a crucial aspect for targeted delivery to the inflamed oral mucosa (Wang et al., 2020). This research seeks to formulate, develop, and evaluate a curcumin-loaded SNEDDS tailored for the management of oral mucositis. The study aims to comprehensively characterize the physicochemical properties of the formulation, assess its in vitro release kinetics, and evaluate its therapeutic efficacy in relevant preclinical models. Through these investigations, we intend to provide valuable insights into the potential of SNEDDS as a delivery platform for enhancing the bioavailability and effectiveness of curcumin in mitigating oral mucositis.

Materials And Methods

MATERIALS

The materials utilized in this study included curcumin (SV Agro Foods, New Delhi; provided as a gift sample), Capryolyl 90, Cremophor EL, and Gelucire 48/16 and (obtained as gift samples from Gattefosse, Mumbai). Ethanol was procured from Merck's Chemicals (Pvt) Ltd., Germany. Tween-80 and acetic acid were obtained from CDH, New Delhi. HPLC grade acetonitrile and methanol from CDH, New Delhi, were used for the preparation of formulations and analytical procedures, along with other analytical grade reagents.

METHODS

Solubility studies

To investigate the solubility of curcumin (CUR), experiments were conducted using various oils, 50% (w/w) surfactant solutions, and co-surfactants. An excess amount of CUR (2 g) was introduced into each selected vehicle and subjected to continuous shaking in a thermostatically controlled water bath for 72 hours at 37 ± 1 ◦C to reach equilibrium. After reaching equilibrium, the mixture underwent centrifugation at 15000 rpm for 20 minutes and then filtered through a 0.45 µ filter membrane. The resulting filtrate was appropriately diluted, and the concentration of CUR was determined at 428 nm using a UV-visible spectrophotometer. Previous studies have noted that CUR exhibits absorbance maxima around 430 nm (Mondal et al., 2016; Priyadarsini, 2009). Each trial was conducted in triplicate. The oils tested included Labrafac PG, Labrafac Lipophile WL 1349, Caprylol 90, Capmul MCM C8, and Acconon MC8-2. The selection of surfactants and co-surfactants comprised Labrasol, Tween 80, Cremophor EL, Transcutol HP, and Gelucire 48/16 (Schmied et al., 2021).

Preliminary screening of surfactants for their emulsifying ability

300 mg of the chosen oil phase was combined with 300 mg of surfactant. This mixture was gently heated to 45–60 °C to facilitate homogenization of the components. A portion of the resulting isotropic mixture (50 mg) was precisely weighed and then diluted with double distilled water to a total volume of 50 mL, creating a fine emulsion. The formation of the emulsion was monitored by counting the number of inversions of a volumetric flask needed to achieve a uniform emulsion. Visual observation was used to assess the relative turbidity of the emulsions. After allowing the emulsions to stand for 2 hours, their transmittance was measured at 638.2 nm using a UV-160A double beam spectrophotometer (Shimadzu, Japan), with double distilled water as the blank (Pattewar et al., 2018).

Preliminary screening of co-surfactants for their emulsifying ability

The turbidimetric method was employed to evaluate co-surfactants' effectiveness in enhancing the nano-emulsification ability of surfactants (Tween 80 and Cremophor EL). In this method, 0.2 grams of surfactants were combined with 0.1 grams of co-surfactant, and the mixture was homogenized using gentle heat (45–60 °C) and vortexing. A portion of the resulting isotropic mixture (50 mg) was precisely weighed and then diluted to 50 mL with double distilled water to create a fine emulsion. The ease of emulsion formation was determined by counting the number of flask inversions required to achieve a uniform emulsion. Visual observation was used to assess the relative turbidity of the emulsions. After allowing the emulsions to stand for 2 hours, their transmittance was measured at 638.2 nm using a UV-160A double beam spectrophotometer (Shimadzu, Japan), with double distilled water as the blank. Since the ratio of co-surfactants to surfactants remained constant, the turbidity of the resulting nanoemulsion was used to evaluate the relative effectiveness of the co-surfactants in enhancing the nano-emulsification ability of the surfactants (Nasr et al., 2016).

Construction of ternary phase diagram

A ternary phase diagram was developed for three-component mixtures comprising oil (Capryol 90 and Capmul MCM C8), surfactant (Cremophor EL), and co-surfactant (Gelucire 48/16) at ambient temperature (25 °C) using the water titration method. Pseudo-ternary diagrams were then created with varying ratios of surfactant to co-surfactant (3:1, 2:1, and 1:1). Different volume ratios of oil to surfactant/co-surfactant (Smix) were thoroughly mixed (1:9, 2:8, 3:7, 4:6, 5:5, 4:6, 3:7, 2:8, 9:1) and titrated with dropwise addition of water under sonication. Each weight ratio of oil and Smix

underwent a slow aqueous titration, and resulting samples that appeared clear or slightly bluish were identified as nanoemulsions. Ternary plots were generated using Chemix software, and these experiments were conducted in triplicate. Self-emulsifying ability was also evaluated using infinite dilution and purified water. Additionally, the impact of CUR on the self-emulsifying performance of SNEDDS was investigated by adding the formulation amount (4% w/w) to the boundary composition of the self-emulsifying domain on the ternary phase diagram (B. Singh et al., 2014).

Optimisation of curcumin nanoemulsion

Multiple SNEDDS formulations with varied compositions were chosen based on the pseudo-ternary phase diagram, focusing on the region with maximum coverage in the 3:1 (Smix) ratio. The phase diagrams encompassed nearly the full range of nanoemulsion (NE) occurrence, with specific oil compositions selected to maintain minimum surfactant concentrations indicative of SNEDDS presence. A Box-Behnken design with 3 central replicates was utilized to optimize the formulation (Detholia et al., 2023; Yadav et al., 2020). The formulation and process variables identified as influential in achieving desired SNEDDS characteristics included the proportion of oil, Smix, and sonication time. These variables primarily impacted crucial factors such as droplet size, polydispersity index (PDI), and percent transmittance, which directly influence the overall quality and performance of the product.

To prepare the drug-loaded SNEDDS, the drug was first dissolved in the oil phase. Subsequently, the required amount of Smix was slowly added, followed by a dropwise addition of water until a clear and transparent liquid was achieved. The resulting NE was tightly sealed and stored at room temperature.

Evaluation of the optimized SNEDDS

Droplet size and zeta potential

A volume of SNEDDS containing 40 mg of CUR was introduced into 500 ml of water in a borosilicate glass beaker. The mixture was then stirred using a magnetic stirrer for 10 minutes at a controlled temperature of 37 ± 0.5 °C. To ensure thorough mixing without creating a vortex, a stirring speed of 150 rpm was maintained. The resulting samples were analyzed for droplet size using dynamic light scattering (DLS) technology and for surface charge (zeta potential) using a Zetasizer Nano ZS instrument (Malvern Analytical Ltd., Malvern, UK). The determination was done in triplicate.

Thermodynamic stability studies

The aim of the thermodynamic stability studies was to assess how variations in temperature affected the SNEDDS formulations. This evaluation involved subjecting the SNEDDS formulations to various stress conditions, including three freeze-thaw cycles (−21 and +25 °C) and heating-cooling cycles (4 and 48 °C), with each temperature phase lasting at least 48 hours. Additionally, the SNEDDS formulations underwent centrifugation stress by being centrifuged at 5000 rpm for 20 minutes using a microcentrifuge (Remi, India), and any signs of phase separation or instability issues were noted. Each stability study was conducted in triplicate for accuracy and consistency.

Morphological investigation

The morphology and structure of the nanoemulsion were examined using a transmission electron microscope (TEM), specifically the TOPCON 002B model operating at 200 kV, which offers high point-to-point resolution. A small amount of diluted nanoemulsion, as prepared for the above test, was placed onto a 300-mesh copper grid and allowed to settle for 1 minute. Subsequently, the grid

was inverted, and a drop of phosphor-tungstic acid (PTA) was applied to the grid for 10 seconds. Excess PTA was then removed by blotting with filter paper. The grid was analyzed using an instrument with a magnification of 1550x at 60-80 kV, and images were captured for further examination.

Drug content

The concentration of CUR in the CUR-loaded SNEDDS was assessed by dissolving an amount equivalent to 40 mg of CUR in ethanol to create the nanoemulsion. These samples were then sonicated, and portions were filtered through a 0.11 µm Sartorius filter (Sartorius, AG, Germany). The appropriately diluted samples were analyzed using HPLC with detection at 428 nm. This analysis was conducted three times for each sample. The drug content was calculated using the formula below:

$$\% \text{ drug content} = \frac{\text{Observed drug content}}{\text{Theoretical drug content}} \text{X}100$$

In vitro drug release

An in vitro drug release investigation was carried out using the USP type-II dissolution apparatus with the paddle method set at a rotational speed of 50 rpm. The dissolution medium comprised 900 ml of phosphate buffer (pH 7.4) containing 1% w/v Tween 80 and maintained at a temperature of 37 ± 0.5 °C. At specific time intervals, 5 ml samples were withdrawn and then filtered using a 0.45 µm syringe filter. The released drug quantity was determined by measuring the absorbance at 428 nm using a UV-1800 Spectrophotometer. This study was conducted in triplicate. Tween 80 was used in the medium to maintain sink conditions due to the hydrophobic nature of CUR.

In vivo pharmacokinetic assessment

Animal handling and care

Male Wistar rats weighing between 200-220 grams were utilized for comparing the pharmacokinetics of CUR-loaded S-SNEDDS with CUR API. The experimental protocol was designed following the guidelines of CPCSEA and was approved by the IAEC of YB Chavan College of Pharmacy, Aurangabad. Prior to the study, the animals were allowed free access to filtered water but were required to fast for 12 hours. They were acclimated and housed in plastic cages under standard laboratory conditions with a temperature of 24 ± 1°C, relative humidity of 55 ± 10%, and a 12-hour light/dark cycle. Throughout the acclimatization period, the health status of the animals was closely monitored.

Dosing

Male Wistar rats were divided into three groups, each consisting of six animals. The pharmacokinetic parameters following a single oral dose of CUR-loaded SNEDDS were compared among the groups: Group 1 received CUR-loaded SNEDDS, Group 2 received pure CUR, and Group 3 received plain water. Blood samples of 0.3 mL were withdrawn from the tail vein at specified time points: 1, 2, 4, 6, and 12 hours. To prevent blood clotting, the blood samples were collected in heparinized microcentrifuge tubes. Plasma was obtained by centrifuging the blood samples at 5000 rpm for 10 minutes at 4°C and then stored at -20°C until further analysis.

Plasma analysis

The concentration of CUR was determined utilizing reverse-phase High-Performance Liquid Chromatography (HPLC) employing a Shimadzu model with an RF-10A XL fluorescence detector. An

analytical column, Phenomenex®00G-4252-E0 C18 100A (250mm×4.6mm, 5µm), was utilized for the analysis. CUR was eluted isocratically at a flow rate of 1 mL/min with an injection volume of 20 µL, using a mobile phase composed of acetonitrile, 20 mM acetate buffer at pH 3.0, and methanol in the ratio of 50:20:30 (v/v). Optimization of fluorescence detection was done with excitation and emission wavelengths set at 420 nm and 530 nm, respectively (Bapat et al., 2019).

Pharmacokinetic data analysis

Nonparametric pharmacokinetic data were computed using the PKSolver add-in program for Microsoft Excel, specifically version 2.0. Standard pharmacokinetic parameters for CUR, including Area under the curve from zero to twenty-four hours and from zero to infinity (AUC_{0-24h} and $AUC_{0-\infty}$), maximum concentration after a single dose (C_{max}), time to reach maximum concentration (Tmax), Clearance (Cl), elimination rate constant (K_E), and terminal half-life ($t_{1/2}$), were obtained from plasma concentration-time curves. The relative bioavailability was determined as the ratio of the oral $AUC_{0-\infty}$ for the test formulation (CUR-loaded SNEDDS) to the AUC0-∞ for the reference (CUR API). These values are presented as the mean ± standard deviation (S.D.).

Results And Discussions

Solubility studies

The drug's solubility in oil is a very important criterion for maintaining the solubilized state of API after dilution. In the case of nanoemulsion, there would be a risk of precipitation of the drug in GIT due to decreased solvent capacity if surfactants or co-surfactants play a critical role in solubilization. Hence, selecting the excipients with the highest solubilization characteristic is an important determinant in the self-emulsifying formulation. The solubility of CUR in various excipients has been shown in Figure 1.

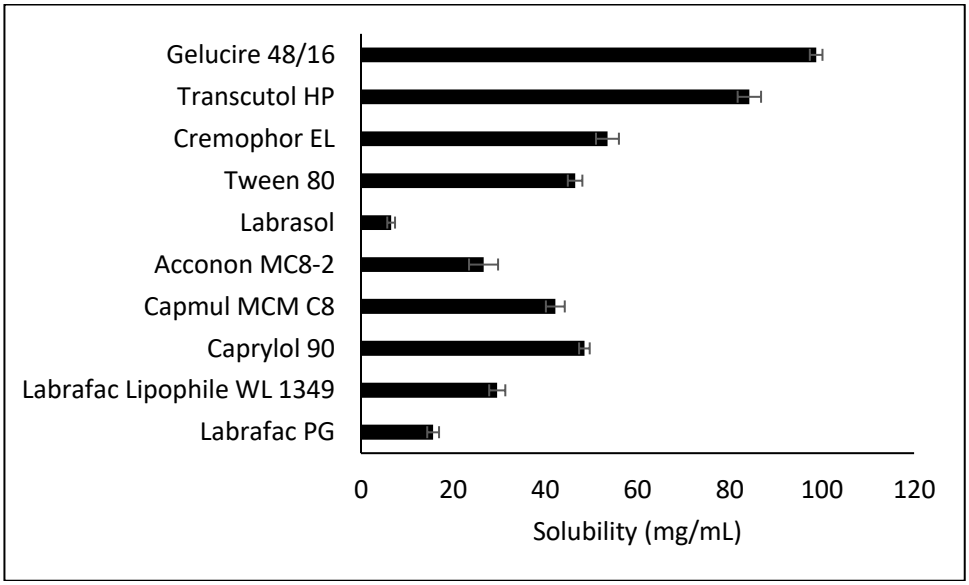

Figure 1: Solubility of CUR in various oils, surfactants, and co-surfactants at 37°C (n=3)

The results, illustrated in Figure 1, indicate that CUR demonstrated solubility in both Caprylol 90 and Capmul MCM C-8, which are the surfactant and emulsifier that were screened. This finding suggests that these are suitable candidates for formulating a CUR-loaded system. Among the surfactants tested, Cremophor EL and Tween 80 exhibited the highest capacity to solubilize CUR. This is an important finding because the surfactants play a crucial role in forming stable

nanoemulsions. Cremophor EL and Tween 80 are known for their ability to enhance the solubility of poorly water-soluble compounds like CUR. This could be attributed to their surfactant properties, which allow them to form micelles, encapsulating the hydrophobic CUR molecules and facilitating their dispersion in the aqueous medium. In contrast, the co-surfactants studied also displayed significant potential to solubilize the drug. Co-surfactants are often used in combination with surfactants to improve the stability and efficiency of nanoemulsions. Their ability to solubilize CUR contributes to the overall efficacy of the nanoemulsion formulation.

These findings are consistent with previous studies in the literature, where Cremophor EL and Tween 80 have been widely recognized for their ability to enhance the solubility and bioavailability of hydrophobic drugs (Khan et al., 2015). Additionally, the successful solubilization of CUR by the co-surfactants suggests that they can play a complementary role in the formulation, further enhancing the stability and performance of the CUR-loaded system.

Overall, the selection of Caprylol 90 and Capmul MCM C-8 , along with Cremophor EL and Tween 80, supported by the co-surfactants' ability to solubilize CUR, lays a strong foundation for the development of an effective and stable CUR-loaded SNEDDS formulation.

Preliminary screening of surfactants for their emulsifying ability

The %transmittance (%T) values of the dispersions prepared using the two shortlisted surfactants are given in Table 1.

Table 1: Emulsification efficiency of Cremophor EL and Tween 80 in Caprylol 90 and Capmul MCM C8

Surfactant	Caprylol 90		Capmul MCM C8	
	%Transmittance (n=3)	No. of inversions	%Transmittance (n=3)	No. of inversions
Tween 80	89.51 ± 0.23	12.5 ± 1.5	85.64 ± 0.41	15.5 ± 1.5
Cremophor EL	97.29 ± 0.33	8.5 ± 1.2	92.58 ± 0.37	11.33 ± 1.5

The %transmittance values in Table 1 provide insights into the emulsifying efficiency of the two shortlisted surfactants, Cremophor EL and Tween 80 when used with Caprylol 90 and Capmul MCM C8 oils. %Transmittance is a measure of the clarity or transparency of an emulsion, with higher values indicating better clarity. Cremophor EL showed a higher %transmittance of 97.29 ± 0.33 with Caprylol 90 compared to Tween 80, suggesting greater efficiency in forming a clear emulsion with this oil. For Capmul MCM C8, Cremophor EL also exhibited a higher %transmittance of 92.58 ± 0.37 compared to Tween 80, indicating its superior ability to create a clear emulsion with this oil. The number of inversions required for emulsification reflects stability, with Cremophor EL generally showing lower values (8.5 ± 1.2 for Caprylol 90 and 11.33 ± 1.5 for Capmul MCM C8) compared to Tween 80 (12.5 ± 1.5 for Caprylol 90 and 15.5 ± 1.5 for Capmul MCM C8). These differences can be attributed to the molecular structures and properties of the surfactants; Cremophor EL forms smaller micelles, resulting in clearer emulsions, while Tween 80 forms larger micelles, which may scatter light more. The findings suggest that Cremophor EL holds promise for the emulsification of CUR-loaded SNEDDS, particularly with Caprylol 90, due to its higher %transmittance values and lower number of inversions required, indicating clear and stable emulsions. Various other researchers have reported similar findings (Croy & Kwon, 2005; Zeng et al., 2017)

Preliminary screening of co-surfactants for their emulsifying ability

The addition of a co-surfactant to the formulation containing surfactant has been reported to improve nanoemulsification and dispersibility (Khan et al., 2012). Co-surfactants were selected based on %transparency and ease of emulsification, as depicted in Table 2.

Table 2: Emulsification studies on surfactant/co-surfactant combinations

Surfactant	Transcutol HP		Gelucire 48/16	
	%Transmittance	No. of inversions	%Transmittance	No. of inversions
Tween 80	86.97 ± 0.45	24.7 ± 3.8	89.51 ± 0.23	12.5 ± 1.5
Cremophor EL	87.48 ± 0.17	19.6 ± 1.6	97.29 ± 0.33	8.5 ± 1.2

Table 2 presents the results from the preliminary screening of co-surfactants for their emulsifying ability in preparing SNEDDS of CUR. %Transmittance values indicate the clarity or transparency of the resulting emulsions, while the number of inversions needed reflects emulsion stability. When combined with Transcutol HP, both Tween 80 and Cremophor EL exhibited similar %transmittance values, with no significant difference observed. However, Cremophor EL required fewer inversions (19.6 ± 1.6) compared to Tween 80 (24.7 ± 3.8), indicating potentially better stability. On the other hand, when paired with Gelucire 48/16, Cremophor EL demonstrated notably higher %transmittance (97.29 ± 0.33) compared to Tween 80 (89.51 ± 0.23), suggesting superior clarity. Additionally, Cremophor EL required fewer inversions (8.5 ± 1.2) than Tween 80 (12.5 ± 1.5), indicating a potentially more stable emulsion. These results suggest that the combination of Cremophor EL as the surfactant with Gelucire 48/16 as the co-surfactant may be the most effective for CUR-loaded SNEDDS, as it exhibited higher %transmittance values and lower number of inversions required, indicating clearer and potentially more stable emulsions. The observed differences can be attributed to the molecular structures and properties of the co-surfactants, which influence their interactions with the surfactant and oil components to form stable nanoemulsions.

Construction of ternary phase diagram

The pseudo-ternary phase diagram, generated using Chemix software, provided a visual representation of the Smix ratios (surfactant:co-surfactant) in combination with Caprylol 90 or Capmul MCM C8 oil and the aqueous phase. This diagram, as shown in Figure 2, is crucial for identifying and optimizing the W/O nanoemulsion region. Pseudo-ternary phase diagrams were constructed for Smix ratios of 1:1, 2:1, and 3:1 individually to precisely pinpoint the nanoemulsion region. These diagrams serve to illustrate the complex relationship between the surfactant mixture and the other components, aiding in the formulation optimization process.

In the Smix ratio of 1:1, the nanoemulsion area appeared narrow, indicating limited stability and potential for nanoemulsion formation. However, as the Smix ratio increased to 2:1, there was a noticeable expansion in the nanoemulsion area. This phenomenon is likely attributed to the further reduction of interfacial tension between oil and water phases, leading to increased fluidity of the interface and thereby enhancing the entropy of the system. Consequently, the larger nanoemulsion region observed at the Smix ratio of 3:1 signifies a higher number of transparent and clear nanoemulsions, indicating improved stability and potential efficacy.

Using the constructed phase diagram, optimal ratios of components for nanoemulsion formation were identified, aiming for formulations that would remain stable and prevent drug precipitation upon infinite dilution. Both Caprylol 90 and Capmul MCM C8 oils displayed a similar trend in nanoemulsion formation, yet the region for the formation of SNEDDS was wider for Caprylol 90

compared to Capmul MCM C8. This observation led to the selection of Caprylol 90 as the preferred oil phase for the preparation of CUR-loaded SNEDDS due to its wider region for stable nanoemulsion formation. This decision is supported by the phase diagram's depiction of a larger area conducive to stable nanoemulsion formation with Caprylol 90, indicating its suitability for maintaining stability and preventing drug precipitation in the SNEDDS formulation.

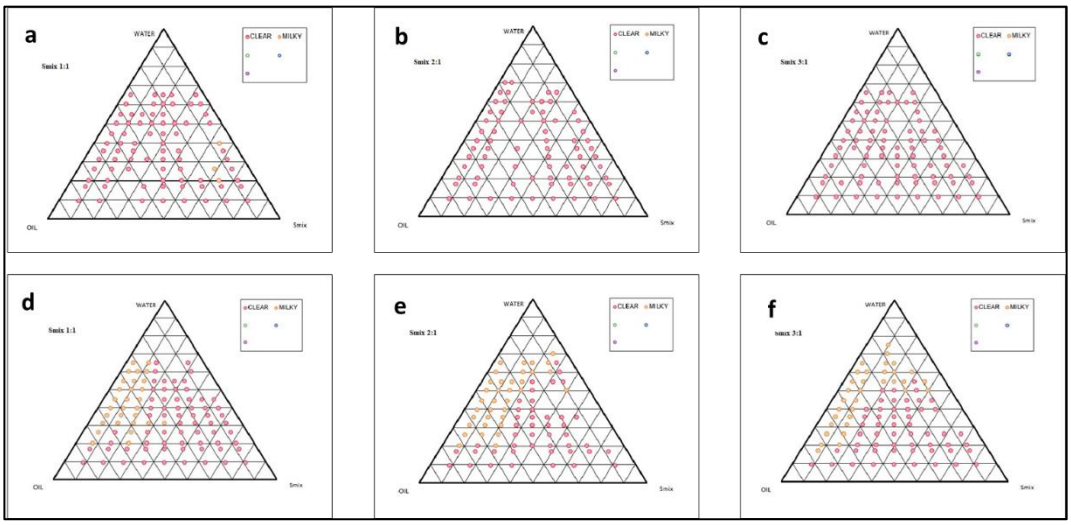

Figure 2: Pseudo ternary phase diagram of a) Smix 1:1 with Caprylol 90, b) Smix 2:1 with Caprylol 90, c) Smix 3:1 with Caprylol 90, d) Smix 1:1 with Capmul MCM C8, e) Smix 2:1 with Capmul MCM C8, and f) Smix 3:1 with Capmul MCM C8

Optimization study

The final optimization was conducted using Design Expert® software version 13 by Stat-Ease, Minneapolis, USA using Caprylol 90 (A) (as oil), Smix (B) (consisting of Cremophor EL, as a surfactant and Gelucire 48/16 as a co-surfactant), and the sonication time (C) as independent variables. The size of droplets (measured in nanometers), PDI, and percentage of transmittance were taken as dependent variables. Responses to the runs, as proposed by the Box-Behnken design, were used to determine the significant model. The polynomial equations obtained for droplet size, PDI, and transmittance are as follows:

Droplet size = $184.67 + 55.125A - 27B - 8.375C - 13.25AB + 0AC + 0.75BC + 29.91A^2 + 4.67B^2 - 0.083C^2$

PDI = $0.271 + 0.10175A - 0.039B - 0.0335C - 0.012AB + 0.0095AC - 0.0085\ BC + 0.07875A^2 + 0.02475B^2 + 0.01625C^2$

% Transmittance = $97.69 - 8.07875A + 3.605B + 2.32625C + 0.532AB - 0.79AC + 0.0825BC - 13.899A^2 - 3.141B^2 - 2.859\ C^2$

Effect of Independent Variables on Droplet Size

Droplet size is a crucial parameter affecting drug release and bioavailability in nanoemulsions. The observed increase in droplet size from 140 nm to 325 nm with varying independent variables provides valuable insights into the formulation process. The significant positive effect of the amount of oil on droplet size is evident, aligning with the basic principles of nanoemulsion formation. A larger amount of oil leads to larger droplets due to increased phase volume. Conversely, the negative effect of Smix concentration on droplet size is expected. As the Smix concentration increases, the surface tension between the oil and aqueous phase decreases, promoting smaller droplet formation.

This phenomenon is supported by the 3D response curves in Figure 3. Furthermore, the decrease in droplet size with an increase in sonication time underscores the role of sonication in breaking down larger droplets into smaller, more uniform ones. This optimization study provides a clear understanding of how these variables interact to influence droplet size, crucial for controlling drug release kinetics and enhancing bioavailability.

Effect of Independent Variables on PDI

The Polydispersity Index (PDI) reflects the uniformity or homogeneity of a formulation, influencing its stability and performance. The observed increase in PDI with increasing lipid concentration and sonication time suggests a wider range of droplet sizes within the nanoemulsion. This can be attributed to the potential for increased agglomeration or incomplete droplet size reduction with higher lipid content and longer sonication times. Conversely, the inverse effect of Smix concentration on PDI, as depicted in Figure 3, indicates that higher Smix concentrations contribute to a more uniform droplet size distribution. This aligns with the role of surfactants in nanoemulsions, where they stabilize droplets and prevent aggregation. The observed PDI values ranging from 0.22 to 0.521 indicate variations in droplet size distribution within the CUR-loaded SNEDDS. These findings emphasize the importance of optimizing Smix concentrations and sonication times to achieve a more uniform and stable nanoemulsion system.

Effect of Independent Variables on Transmittance

Transmittance is a crucial indicator of the transparency and stability of a formulation. The observed range of transmittance values (67.92% to 97.8%) reflects the varying degrees of formulation clarity. The negative impact of oil percentage on transmittance is logical, as higher oil concentrations can lead to increased light scattering and reduced transparency. Conversely, the slight positive effect of Smix and sonication time on transmittance indicates their roles in promoting formulation clarity. Higher transmittance values signify a more transparent and stable formulation, which is desirable for drug delivery systems. The correlation between the independent variables and transmittance, as illustrated in Figure 3, highlights the importance of balancing these factors to achieve an optimal formulation with desired transparency and stability.

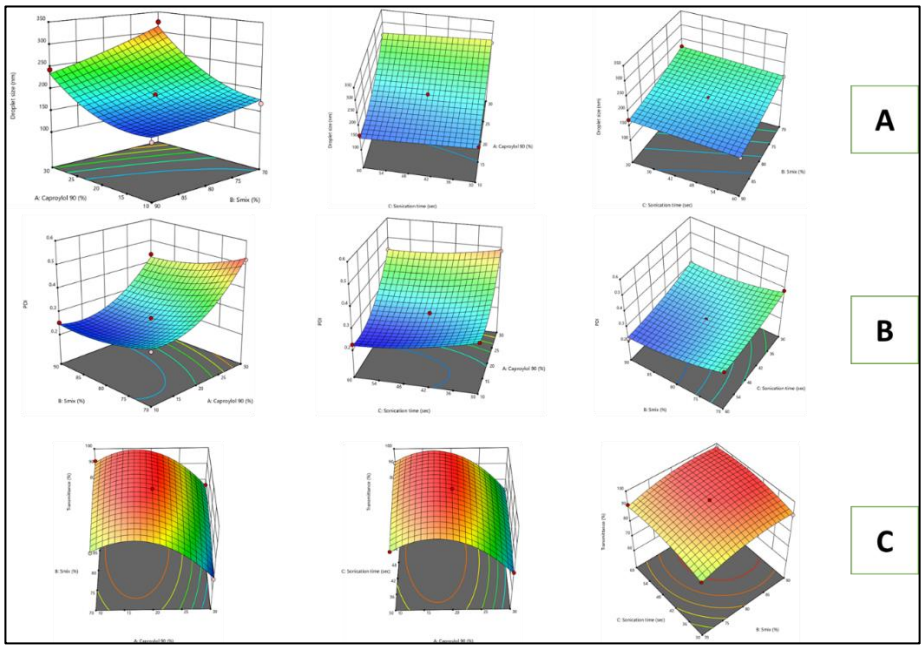

Figure 3: The 3D surface response plot showing the effect of independent variables (Oil, Smix, and Sonication time) on dependent variables (A) droplet size, (B) PDI, and (C) % transmittance

Validation and Point Prediction

The validation and point prediction phase of the optimization study aimed to assess the accuracy of the generated model in predicting an optimal formulation. The software predicted an optimal formulation composition of 19.6% oil, 80.5% Smix, and 51 seconds of sonication time, with predicted responses for droplet size, PDI, and transmittance. The observed values closely matched the predicted values, as seen in Figure 4, indicating the robustness and accuracy of the optimization process. The high degree of correlation between predicted and observed values validates the model's efficacy in optimizing the formulation and process parameters for CUR-loaded SNEDDS. This validation step ensures confidence in the selected formulation, demonstrating its potential for stable and efficient drug delivery applications.

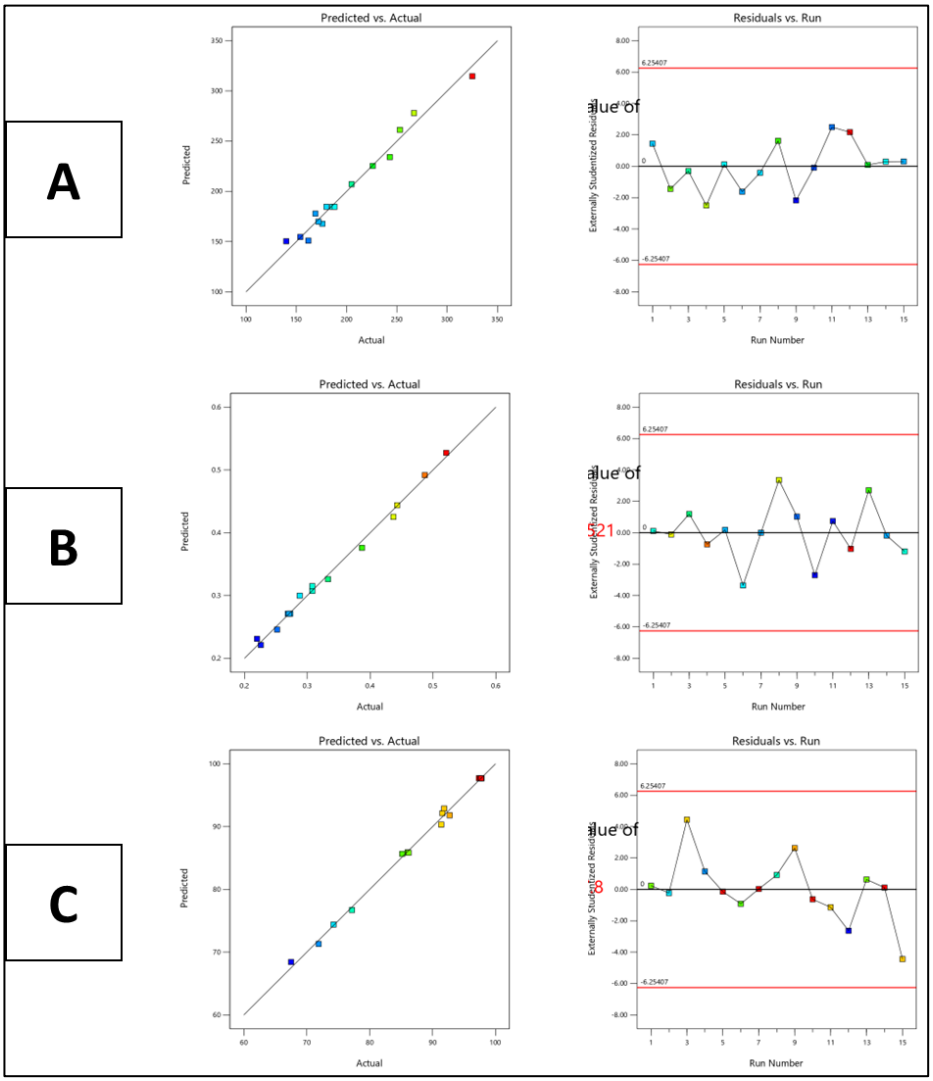

Figure 4: Linear correlation plot between observed and predicted values of a) Droplet size, B) PDI and c) % Transmittance and their corresponding residual plots

Evaluation of optimized liquid SNEDDS

Droplet size and zeta potential

The average globule size of the formulated nanoemulsion, measured at 181.3 ± 6.5 nm (Figure 5) after drug loading, provides crucial insights into the physical characteristics of the system. This size

is within the desirable range for nanoemulsions, which is less than 200 nm (Buddhadev et al., 2024). Additionally, the low PDI value of 0.27 is indicative of a narrow size distribution among the globules. A PDI value closer to zero signifies uniformity in particle size distribution, meaning the majority of globules are similar in size. For lipid-based formulations, a PDI value lesser than 0.3 is considered acceptable (Reddy & Gubbiyappa, 2022). This uniformity is advantageous for drug delivery systems, as it ensures consistent and predictable behavior of the nanoemulsion.

Moreover, the negative zeta potential of -11.7 mV observed in the nanoemulsion is significant for its stability. Zeta potential reflects the electrostatic repulsion between particles, with higher absolute values indicating greater repulsion and, thus, enhanced stability. In this case, the negative zeta potential suggests that the globules possess anionic surface charges, contributing to their stability by preventing aggregation or coalescence. The relatively moderate magnitude of the zeta potential (-11.7 mV) is favorable, as it indicates sufficient stability without excessive repulsion that could lead to flocculation or sedimentation. Caprylol 90 is a medium-chain triglyceride derived from caprylic acid, and Gelucire 48/16 is a mixture of glycerides and polyethylene glycol (PEG) esters of fatty acids. The presence of fatty acid esters in these components used to formulate the SNEDDS would have led to the development of a negative value of zeta potential (Rathore et al., 2023).

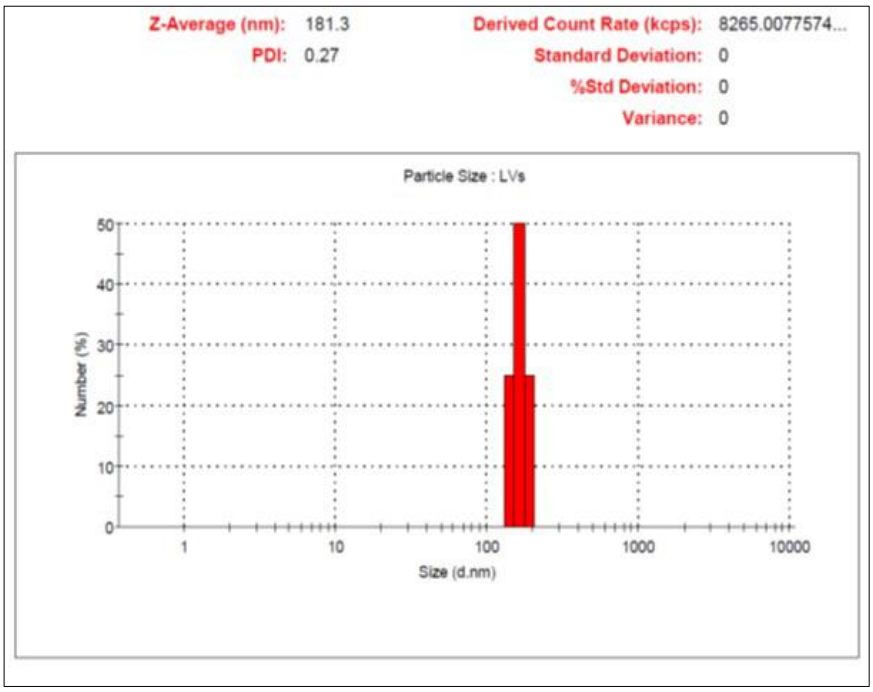

Figure 5: Particle size distribution of the CUR-loaded SNEDDS

Thermodynamic stability studies

The thermodynamic stability studies conducted on the optimized CUR-loaded SNEDDS revealed promising results, as the formulation displayed remarkable resilience against various high-stress conditions. The absence of precipitation, cracking, creaming, or turbidity, even after exposure to rigorous centrifugation and repeated freeze-thaw and heating-cooling cycles, indicates the robust stability of the SNEDDS formulation. This stability is crucial for pharmaceutical formulations, as it ensures that the drug remains uniformly dispersed and accessible for absorption upon administration. The observed resistance to physical and temperature-induced stress can be attributed to the balanced composition of the SNEDDS, where the surfactant and co-surfactant components provide a protective layer around the oil droplets, preventing coalescence or separation

(Zembyla et al., 2020). Additionally, the small globule size and narrow size distribution contribute to the formulation's stability by minimizing interactions that could lead to instability (H. Singh et al., 2019).

Morphological investigation

The examination of the morphology of the optimized CUR-loaded SNEDDS using TEM provided valuable insights into the nanoemulsion structure. The TEM images revealed discrete, spherical oil globules with diameters ranging from 0.16 to 0.2 µm, as depicted in Figure 6. The dark appearance of the oil globules against bright surroundings indicates a contrast typical of TEM imaging, where dense structures appear dark. The presence of non-aggregated globules suggests excellent physical stability of the nanoemulsion (Ke et al., 2016; Parmar et al., 2015). The observed spherical morphology is favorable for drug delivery systems, as it offers a large surface area for drug release and absorption, enhancing bioavailability. The uniform size distribution, as indicated by the consistent globule diameters, further supports the formulation's stability and uniformity. These findings are consistent with a well-optimized SNEDDS formulation, where the surfactant and co-surfactant components effectively stabilize the oil globules.

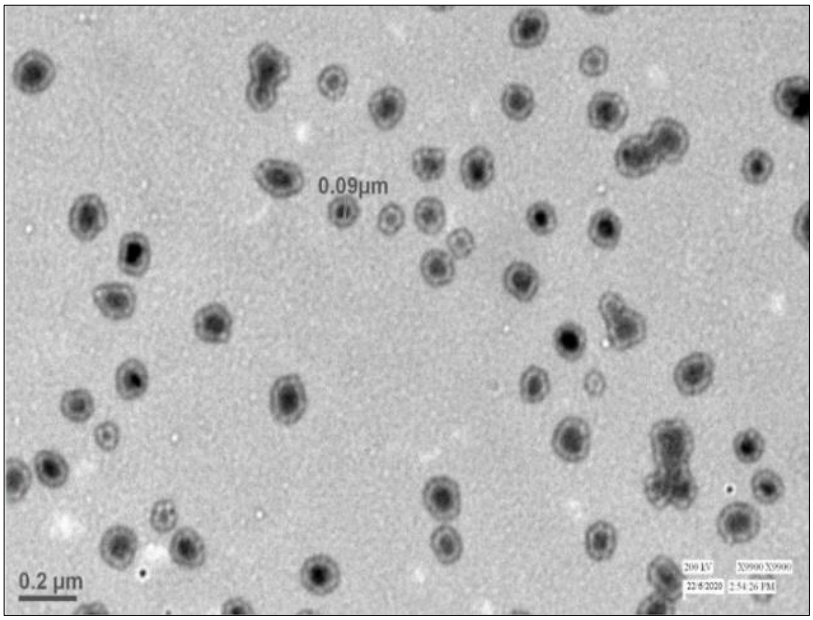

Figure 6: TEM scan of the prepared CUR-loaded SNEDDS

Drug content

The analysis of drug content in the nanoemulsion revealed an impressive value of 98.2 ± 1.64%. This high drug content indicates the efficient loading of the drug within the nanoemulsion formulation, ensuring that a significant amount of the drug is present in the final product. The exceptional drug content percentage further confirms the stability and uniformity of the nanoemulsion, suggesting minimal drug loss during the formulation process.

In vitro drug release

Various researchers have used Tween 80 in the dissolution medium ranging from 0.1 to 10%, but on conducting trials, we observed that a concentration of 1 % w/v Tween 80 was sufficient to maintain sink conditions (Shahani & Panyam, 2011; Wei et al., 2020). A similar concentration of Tween 80 has been employed by Maria et al., who had prepared an inclusion complex of CUR with

cyclodextrin (Maria et al., 2017). The in vitro drug release investigation using USP type-II dissolution apparatus with a paddle method revealed significant differences between the release profiles of CUR from its powder form and the optimized SNEDDS formulation, as depicted in Figure 7. The results demonstrate that the CUR-loaded SNEDDS exhibited a remarkably higher drug release, with over 85% of the drug released within 2 hours, compared to just 25% of the drug released from its native state. This substantial enhancement in drug release from the SNEDDS can be attributed to several factors. Firstly, the nanoemulsion formulation provides a large interfacial surface area, facilitating rapid d/rug dissolution and release. Secondly, the presence of surfactants, such as Cremophor EL and Gelucire 48/16, in the SNEDDS formulation aids in solubilizing CUR, known for its hydrophobic nature, thereby enhancing its availability for release. Thirdly, the uniform and stable nature of the nanoemulsion, as observed in the TEM analysis, ensures consistent and rapid drug release. The use of Tween 80 in the dissolution medium maintained sink conditions, ensuring that the concentration of the dissolved drug remains significantly lower than its solubility. This study highlights the efficacy of the optimized SNEDDS formulation in achieving a rapid and efficient drug release profile, which is essential for improving CUR's bioavailability and therapeutic efficacy.

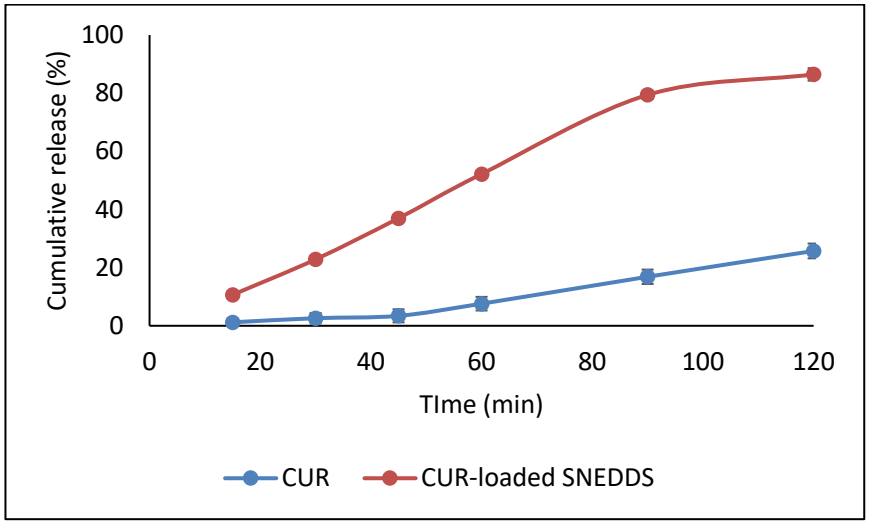

Figure 7: In vitro drug release profile of plain CUR and CUR-loaded SNEDDS

In vivo pharmacokinetic assessment

The plasma concentration-time profile of plain CUR and CUR-loaded SNEDDS is given in Figure 8 below.

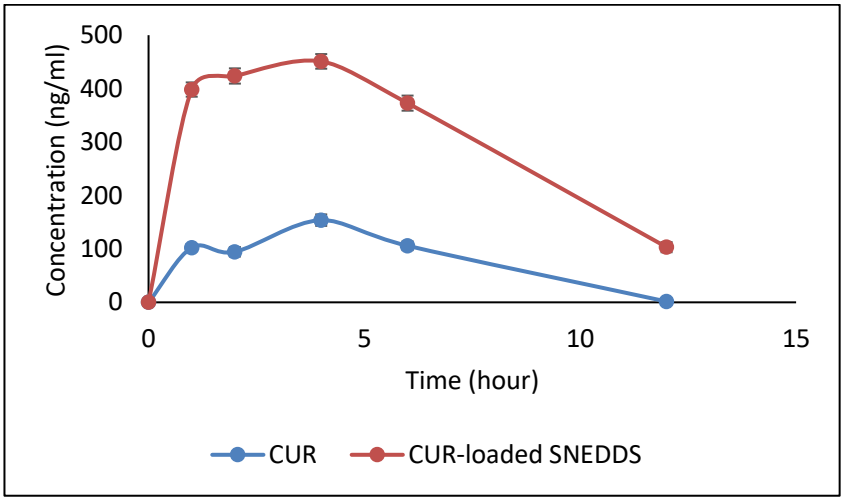

Figure 8: Plasma concentration-time profile of plain CUR and CUR-loaded SNEDDS

The pharmacokinetic assessment comparing plain CUR and CUR-loaded SNEDDS reveals significant improvements in several key parameters with the nanoemulsion formulation. The most notable enhancement is seen in the maximum plasma concentration (C_{max}) of CUR-loaded SNEDDS, reaching 364.861 ng/ml compared to 153.082 ng/ml for plain CUR. This substantial increase in Cmax indicates improved bioavailability, allowing for higher drug concentrations to be reached in the bloodstream upon administration. The longer half-life ($t_{1/2}$) of 2.335 hours for CUR-loaded SNEDDS, compared to 1.779 hours for plain CUR, suggests a prolonged retention time in the body, potentially leading to sustained therapeutic effects. The area under the concentration-time curve from zero to infinity ($AUC_{0-\infty}$) also demonstrates a substantial increase from 930.915 ng.h/ml for plain CUR to 2686.403 ng.h/ml for CUR-loaded SNEDDS, further confirming enhanced drug exposure and systemic availability. These improvements can be attributed to the nanoemulsion's ability to solubilize and protect CUR, facilitating its absorption and circulation in the bloodstream. The relatively low Cl/F_obs value of 0.009 (mg)/(ng/ml)/h for CUR-loaded SNEDDS compared to 0.026 (mg)/(ng/ml)/h for plain CUR indicates reduced clearance, supporting the notion of prolonged systemic exposure and sustained release. The $MRT_{0-\infty}$ value of 4.99 hours for CUR-loaded SNEDDS also suggests an extended duration of drug presence in the body. The observed parameters collectively suggest that the optimized SNEDDS formulation significantly improves the pharmacokinetic profile of CUR, enhancing its bioavailability and potential therapeutic efficacy. These findings underscore the potential of CUR-loaded SNEDDS as a promising drug delivery system for improving the treatment outcomes of CUR.

Conclusion

The comprehensive study on the development and optimization of CUR-loaded Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) presents a promising platform for enhancing the bioavailability and therapeutic efficacy of curcumin. The initial solubility studies highlighted Caprylol 90 and Capmul MCM C-8 oils, along with Cremophor EL and Tween 80 surfactants, as ideal candidates for formulating the SNEDDS. Subsequent evaluations of emulsifying abilities revealed Cremophor EL to be superior, particularly when paired with Gelucire 48/16 as a co-surfactant. The construction of a pseudo-ternary phase diagram further guided the selection of Caprylol 90 as the preferred oil phase due to its wider nanoemulsion region. The optimization study using Design Expert® software validated the robustness of the formulation, with observed and predicted responses closely aligned. The optimized CUR-loaded SNEDDS exhibited desirable physical properties, including a small globule size (181.3 $\pm$ 6.5 nm) with a narrow size distribution (PDI of 0.27) and a negative zeta potential (-11.7 mV) indicative of stability. Thermodynamic stability studies confirmed the formulation's resilience to stress conditions, while morphological analysis via TEM revealed uniform and stable oil globules. The formulation achieved an impressive drug content of 98.2 $\pm$ 1.64%, ensuring efficient drug loading. Importantly, the in vitro drug release study demonstrated a substantial enhancement in drug release (over 85% within 2 hours) compared to plain CUR, attributed to the nanoemulsion's properties. Pharmacokinetic assessment in vivo revealed significant improvements in C_{max}, AUC, $t_{1/2}$, and MRT for CUR-loaded SNEDDS, indicating enhanced bioavailability and prolonged systemic exposure. These findings collectively highlight the potential of the optimized CUR-loaded SNEDDS as an effective drug delivery system for improving the therapeutic outcomes of curcumin, offering a promising avenue for future research and clinical applications in the treatment of various diseases.

References

1. Abushal, A. S., Aleanizy, F. S., Alqahtani, F. Y., Shakeel, F., Iqbal, M., Haq, N., & Alsarra, I. A. (2022). Self-Nanoemulsifying Drug Delivery System (SNEDDS) of Apremilast: In Vitro Evaluation and Pharmacokinetics Studies. Molecules, 27(10), Article 10. https://doi.org/10.3390/molecules27103085
2. Baloch, J., Sohail, M. F., Sarwar, H. S., Kiani, M. H., Khan, G. M., Jahan, S., Rafay, M., Chaudhry, M. T., Yasinzai, M., & Shahnaz, G. (2019). Self-Nanoemulsifying Drug Delivery System (SNEDDS) for Improved Oral Bioavailability of Chlorpromazine: In Vitro and In Vivo Evaluation. Medicina, 55(5). https://doi.org/10.3390/medicina55050210
3. Bapat, P., Ghadi, R., Chaudhari, D., Katiyar, S. S., & Jain, S. (2019). Tocophersolan stabilized lipid nanocapsules with high drug loading to improve the permeability and oral bioavailability of curcumin. International Journal of Pharmaceutics, 560, 219-227. https://doi.org/10.1016/j.ijpharm.2019.02.013
4. Buddhadev, S. S., C. Garala, K., S, S., Rahamathulla, M., Ahmed, M. M., Farhana, S. A., & Pasha, I. (2024). Quality by design aided self-nano emulsifying drug delivery systems development for the oral delivery of Benidipine: Improvement of biopharmaceutical performance. Drug Delivery, 31(1), 2288801. https://doi.org/10.1080/10717544.2023.2288801
5. Chopra, H., Dey, P. S., Das, D., Bhattacharya, T., Shah, M., Mubin, S., Maishu, S. P., Akter, R., Rahman, Md. H., Karthika, C., Murad, W., Qusty, N., Qusti, S., Alshammari, E. M., Batiha, G. E.-S., Altalbawy, F. M. A., Albooq, M. I. M., & Alamri, B. M. (2021). Curcumin Nanoparticles as Promising Therapeutic Agents for Drug Targets. Molecules, 26(16), 4998. https://doi.org/10.3390/molecules26164998
6. Croy, S. R., & Kwon, G. S. (2005). Polysorbate 80 and Cremophor EL micelles deaggregate and solubilize nystatin at the core-corona interface. Journal of Pharmaceutical Sciences, 94(11), 2345-2354. https://doi.org/10.1002/jps.20301
7. De Leo, V., Milano, F., Mancini, E., Comparelli, R., Giotta, L., Nacci, A., Longobardi, F., Garbetta, A., Agostiano, A., & Catucci, L. (2018). Encapsulation of Curcumin-Loaded Liposomes for Colonic Drug Delivery in a pH-Responsive Polymer Cluster Using a pH-Driven and Organic Solvent-Free Process. Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry, 23(4), 739. https://doi.org/10.3390/molecules23040739
8. Detholia, K., Mohandas, A., Varia, U., Jadeja, M., & Katariya, H. (2023). Development and optimization of Ropinirole loaded self-nanoemulsifying tablets. Future Journal of Pharmaceutical Sciences, 9(1), 66. https://doi.org/10.1186/s43094-023-00516-x
9. Gholipour, F., Amini, M., Baradaran, B., Mokhtarzadeh, A., & Eskandani, M. (2023). Anticancer properties of curcumin-treated Lactobacillus plantarum against the HT-29 colorectal adenocarcinoma cells. Scientific Reports, 13(1), Article 1. https://doi.org/10.1038/s41598-023-29462-7
10. Górnicka, J., Mika, M., Wróblewska, O., Siudem, P., & Paradowska, K. (2023). Methods to Improve the Solubility of Curcumin from Turmeric. Life, 13(1), 207. https://doi.org/10.3390/life13010207
11. Hegde, M., Girisa, S., BharathwajChetty, B., Vishwa, R., & Kunnumakkara, A. B. (2023). Curcumin Formulations for Better Bioavailability: What We Learned from Clinical Trials Thus Far? ACS Omega, 8(12), 10713-10746. https://doi.org/10.1021/acsomega.2c07326

12. Jakubczyk, K., Drużga, A., Katarzyna, J., & Skonieczna-Żydecka, K. (2020). Antioxidant Potential of Curcumin—A Meta-Analysis of Randomized Clinical Trials. Antioxidants, 9(11), 1092. https://doi.org/10.3390/antiox9111092
13. Jiang, L., Xia, N., Wang, F., Xie, C., Ye, R., Tang, H., Zhang, H., & Liu, Y. (2022). Preparation and characterization of curcumin/β-cyclodextrin nanoparticles by nanoprecipitation to improve the stability and bioavailability of curcumin. LWT, 171, 114149. https://doi.org/10.1016/j.lwt.2022.114149
14. Ke, Z., Hou, X., & Jia, X. (2016). Design and optimization of self-nanoemulsifying drug delivery systems for improved bioavailability of cyclovirobuxine D. Drug Design, Development and Therapy, 10, 2049–2060. https://doi.org/10.2147/DDDT.S106356
15. Khan, A. W., Kotta, S., Ansari, S. H., Sharma, R. K., & Ali, J. (2012). Potentials and challenges in self-nanoemulsifying drug delivery systems. Expert Opinion on Drug Delivery, 9(10), 1305–1317. https://doi.org/10.1517/17425247.2012.719870
16. Khan, A. W., Kotta, S., Ansari, S. H., Sharma, R. K., & Ali, J. (2015). Self-nanoemulsifying drug delivery system (SNEDDS) of the poorly water-soluble grapefruit flavonoid Naringenin: Design, characterization, in vitro and in vivo evaluation. Drug Delivery, 22(4), 552–561. https://doi.org/10.3109/10717544.2013.878003
17. Krstić, M., Medarević, Đ., Đuriš, J., & Ibrić, S. (2018). Chapter 12—Self-nanoemulsifying drug delivery systems (SNEDDS) and self-microemulsifying drug delivery systems (SMEDDS) as lipid nanocarriers for improving dissolution rate and bioavailability of poorly soluble drugs. In A. M. Grumezescu (Ed.), Lipid Nanocarriers for Drug Targeting (pp. 473–508). William Andrew Publishing. https://doi.org/10.1016/B978-0-12-813687-4.00012-8
18. Maria, D. N., Mishra, S. R., Wang, L., Abd-Elgawad, A.-E. H., Soliman, O. A.-E., El-Dahan, M. S., & Jablonski, M. M. (2017). Water-soluble Complex of Curcumin with Cyclodextrins: Enhanced Physical Properties For Ocular Drug Delivery. Current Drug Delivery, 14(6), 875–886. https://doi.org/10.2174/1567201813666160808111209
19. Mondal, S., Ghosh, S., & Moulik, S. P. (2016). Stability of curcumin in different solvent and solution media: UV-visible and steady-state fluorescence spectral study. Journal of Photochemistry and Photobiology B: Biology, 158, 212–218. https://doi.org/10.1016/j.jphotobiol.2016.03.004
20. Nasr, A., Gardouh, A., & Ghorab, M. (2016). Novel Solid Self-Nanoemulsifying Drug Delivery System (S-SNEDDS) for Oral Delivery of Olmesartan Medoxomil: Design, Formulation, Pharmacokinetic and Bioavailability Evaluation. Pharmaceutics, 8(3). https://doi.org/10.3390/pharmaceutics8030020
21. Parmar, K., Patel, J., & Sheth, N. (2015). Self nano-emulsifying drug delivery system for Embelin: Design, characterization and in-vitro studies. Asian Journal of Pharmaceutical Sciences, 10(5), 396–404. https://doi.org/10.1016/j.ajps.2015.04.006
22. Pattewar, S., Kasture, S. B., P, V. V., e, Patil, D. N., & Sharma, S. K. (2018). Development and Optimization of Piroxicam-loaded Solid Self-micro emulsifying Drug Delivery System. Indian Journal of Pharmaceutical Sciences, 80(2), 350–358. https://doi.org/10.4172/pharmaceutical-sciences.1000364
23. Priyadarsini, K. I. (2009). Photophysics, photochemistry and photobiology of curcumin: Studies from organic solutions, bio-mimetics and living cells. Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 10(2), 81–95. https://doi.org/10.1016/j.jphotochemrev.2009.05.001

24. Pulito, C., Cristaudo, A., Porta, C. L., Zapperi, S., Blandino, G., Morrone, A., & Strano, S. (2020). Oral mucositis: The hidden side of cancer therapy. Journal of Experimental & Clinical Cancer Research, 39(1), 210. https://doi.org/10.1186/s13046-020-01715-7
25. Ramezani, V., Ghadirian, S., Shabani, M., Boroumand, M. A., Daneshvar, R., & Saghafi, F. (2023). Efficacy of curcumin for amelioration of radiotherapy-induced oral mucositis: A preliminary randomized controlled clinical trial. BMC Cancer, 23, 354. https://doi.org/10.1186/s12885-023-10730-8
26. Rathore, C., Hemrajani, C., Sharma, A. K., Gupta, P. K., Jha, N. K., Aljabali, A. A. A., Gupta, G., Singh, S. K., Yang, J.-C., Dwivedi, R. P., Dua, K., Chellappan, D. K., Negi, P., & Tambuwala, M. M. (2023). Self-nanoemulsifying drug delivery system (SNEDDS) mediated improved oral bioavailability of thymoquinone: Optimization, characterization, pharmacokinetic, and hepatotoxicity studies. Drug Delivery and Translational Research, 13(1), 292-307. https://doi.org/10.1007/s13346-022-01193-8
27. Reddy, M. R., & Gubbiyappa, K. S. (2022). Formulation development, optimization and characterization of Pemigatinib-loaded supersaturable self-nanoemulsifying drug delivery systems. Future Journal of Pharmaceutical Sciences, 8(1), 45. https://doi.org/10.1186/s43094-022-00434-4
28. Schmied, F.-P., Bernhardt, A., Engel, A., & Klein, S. (2021). A Customized Screening Tool Approach for the Development of a Self-Nanoemulsifying Drug Delivery System (SNEDDS). AAPS PharmSciTech, 23(1), 39. https://doi.org/10.1208/s12249-021-02176-7
29. Shahani, K., & Panyam, J. (2011). Highly Loaded, Sustained-Release Microparticles of Curcumin for Chemoprevention. Journal of Pharmaceutical Sciences, 100(7), 2599-2609. https://doi.org/10.1002/jps.22475
30. Shetty, S. S., Maruthi, M., Dhara, V., de Arruda, J. A. A., Abreu, L. G., Mesquita, R. A., Teixeira, A. L., Silva, T. A., & Merchant, Y. (2022). Oral mucositis: Current knowledge and future directions. Disease-a-Month, 68(5), 101300. https://doi.org/10.1016/j.disamonth.2021.101300
31. Singh, B., Beg, S., Khurana, R. K., Sandhu, P. S., Kaur, R., & Katare, O. P. (2014). Recent advances in self-emulsifying drug delivery systems (SEDDS). Critical Reviews in Therapeutic Drug Carrier Systems, 31(2), 121-185. https://doi.org/10.1615/critrevtherdrugcarriersyst.2014008502
32. Singh, H., Nathani, S., Singh, N., Roy, P., Paul, S., Sohal, H. S., & Jain, S. K. (2019). Development and characterization of Solid-SNEDDS formulation of DHA using hydrophilic carrier with improved shelf life, oxidative stability and therapeutic activity. Journal of Drug Delivery Science and Technology, 54, 101326. https://doi.org/10.1016/j.jddst.2019.101326
33. Tønnesen, H. H., Másson, M., & Loftsson, T. (2002). Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: Solubility, chemical and photochemical stability. International Journal of Pharmaceutics, 244(1), 127-135. https://doi.org/10.1016/S0378-5173(02)00323-X
34. Villa, A., & Sonis, S. T. (2016). Pharmacotherapy for the management of cancer regimen-related oral mucositis. Expert Opinion on Pharmacotherapy, 17(13), 1801-1807. https://doi.org/10.1080/14656566.2016.1217993
35. Wang, C.-Y., Yen, C.-C., Hsu, M.-C., & Wu, Y.-T. (2020). Self-Nanoemulsifying Drug Delivery Systems for Enhancing Solubility, Permeability, and Bioavailability of Sesamin. Molecules, 25(14), 3119. https://doi.org/10.3390/molecules25143119

36. Wei, X.-Q., Zhu, J.-F., Wang, X.-B., & Ba, K. (2020). Improving the Stability of Liposomal Curcumin by Adjusting the Inner Aqueous Chamber pH of Liposomes. ACS Omega, 5(2), 1120-1126. https://doi.org/10.1021/acsomega.9b03293
37. Yadav, P., Rastogi, V., & Verma, A. (2020). Application of Box-Behnken design and desirability function in the development and optimization of self-nanoemulsifying drug delivery system for enhanced dissolution of ezetimibe. Future Journal of Pharmaceutical Sciences, 6(1), 7. https://doi.org/10.1186/s43094-020-00023-3
38. Zembyla, M., Murray, B. S., & Sarkar, A. (2020). Water-in-oil emulsions stabilized by surfactants, biopolymers and/or particles: A review. Trends in Food Science & Technology, 104, 49-59. https://doi.org/10.1016/j.tifs.2020.07.028
39. Zeng, L., Xin, X., & Zhang, Y. (2017). Development and characterization of promising Cremophor EL-stabilized o/w nanoemulsions containing short-chain alcohols as a cosurfactant. RSC Advances, 7(32), 19815-19827. https://doi.org/10.1039/C6RA27096D