Journal of Young Pharmacists, 2025; 17(1):166-175.
https://www.jyoungpharm.org

Original Article

Spontaneous Emulsification as a Novel Approach for the Preparation and Characterization of Curcumin Nanoemulsion: Advancing Bioavailability and Therapeutic Efficacy

Joshna Booravilli*, Janaki Devi Sirisolla

Department of Pharmaceutics, GITAM School of Pharmacy, GITAM (Deemed to be University), Rushikonda, Visakhapatnam, Andhra Pradesh, INDIA.

ABSTRACT

Purpose: Curcumin is a phytochemical that is bioactive and exhibits strong therapeutic effects on several skin diseases. Curcumin's (CUR) low water solubility and restricted skin permeability constitute a major obstacle to its topical efficacy. The main objective was to prepare and evaluate a nanoemulsion using curcumin to improve its bioavailability. This work evaluated the impact of various emulsifiers (Cremophor ® RH 40 and PEG 400) and surfactant-to-oil ratios (s/o) on the stability, physical, and chemical characteristics of curcumin-loaded nanoemulsions. Clove oil and the Surfactant to cosurfactant (S_{mix}) ratio of 1:1 were chosen in accordance with the ternary phase diagram's nanoemulsion zone, which illustrates the relationship between the nanoemulsion's phase behavior and composition in order to create a stable nanoemulsion loaded with curcumin. **Materials and Methods:** Curcumin-containing nanoemulsions were obtained through the spontaneous emulsification method. **Results:** Zeta potential, viscosity, particle size, and physical properties of the produced nanoemulsions were evaluated. Thus, Curcumin-containing Nanoemulsions (CUR-CLO NEG 1) stabilized with surfactant and cosurfactant had lower particle sizes and zeta potential of 30.2±0.11 and -38.3±0.23 respectively, thereby improving physical stabilities and increased drug content of 97±0.11%. Due to its decreased oil content, CNE1 has the lowest viscosity. Reduced oil content causes droplet size to decrease; this can be explained by how oil affects nanoemulsions' viscosity (i.e., lower viscosity values lead to reduced oil content). **Conclusion:** The findings from the above research work concluded that the curcumin nanoemulsion formulated using the spontaneous emulsion method produced a uniform and stable nanoemulsion with a small droplet size, which may be suitable for topical drug delivery.

Keywords: Curcumin, Nanoemulsion, Solubility, Spontaneous emulsification, S_{mix}.

Correspondence:
Dr. Janaki Devi Sirisolla
Department of Pharmaceutics,
GITAM School of Pharmacy, GITAM
Deemed to be University, Rushikonda,
Visakhapatnam-530045, Andhra Pradesh,
INDIA.
Email: jsirisol@gitam.edu
ORCID: 0000-0002-6181-8899

Received: 10-08-2024;
Revised: 30-08-2024;
Accepted: 20-10-2024.

INTRODUCTION

The bioavailability of hydrophobic substances has been effectively increased by using formulations based on nanotechnologies, such as niosomes/Proniosomes,[1,2] Solid Lipid Nanoparticles (SLN), drug nanocrystals,[3] liposomes,[4] and Nanostructured Lipid Carriers (NLC).[5,6] This achievement is demonstrated by the US FDA has approval of several formulations based on nanotechnology to treat various illnesses. Therefore, the formulation of nanoemulsions is one of the best approaches to increasing the solubilization and bioavailability of these weakly soluble drugs.

Curcumin is a naturally occurring polyphenolic molecule with numerous pharmacological benefits, including anti-cancer, anti-inflammatory, and antioxidant, properties. However, investigations into the metabolism and absorption of curcumin have demonstrated that, upon delivery, either no curcumin or very little of it was found in serum or tissue. Curcumin's rapid hydrolysis, followed by molecular fragmentation at physiological pH, its limited solubility in aqueous environments, and the inactivity of its metabolic products are thought to be the primary causes of the limited bioavailability.

Due to its weak water solubility, curcumin can only be applied topically via the superficial Stratum Corneum (SC). Curcumin is one example of a lipophilic substance that can boost the dosage at the desired location and deliver curcumin directly into the skin without causing any systemic adverse effects. Therefore, for the efficient and targeted distribution of curcumin, a novel formulation that releases curcumin continuously is needed.[7]

OPEN ACCESS

Manuscript TECHNOMEDIA

DOI: 10.5530/jyp.20251414

Nanoemulsions are a form of heterogeneous colloidal particulate system comprising two or more immiscible liquids dispersed into each other as tiny droplets with a diameter of 20 to 500 nm. They are commonly referred as ultrafine emulsions, submicron emulsions, and mini-emulsions. These are thermodynamically and kinetically stable[7] systems with extremely minute droplet sizes that don't seem to flocculate or coalesce during extended storage times. They consist of an emulsifying agent that can stabilize this thermodynamically unstable system; they are also called emulsifiers or emulgents. While the outer phase is referred to as the dispersion medium, external phase, or continuous phase, the internal phase is also known as the disperse phase or interior phase. The creation of a ternary phase diagram is the first stage in the construction of a nanoemulsion.

A ternary graph is a type of graphic representation used to display the species compositions of systems made up of three or more.[8] This barycentric graphic represents The three variable ratios as locations in an equilateral triangle. The proportions of the three variables (a, b, and c) in a ternary plot must add up to a constant, typically shown as 100%. This indicates that a+b+c=K for all graphed substances, where K is the constant. There are just two degrees of freedom, and the three proportions cannot fluctuate separately; therefore, knowing two variables is enough to locate a sample's point on the graph. For instance, c can be computed as K-a-b provided a and b are known. We may visually show the intersection of all three variables in two dimensions using the ternary plot. Studying the mutual solubilities of liquids in a two-phase system, such as water and oil, in contact with a surfactant is one application for which it is beneficial.[9] Understanding the behavior of the various phases and their components is easier with the ternary plot's aid. The temperature, pressure, and compositions of two of the three elements are the four degrees of freedom that a single phase in a three-component system can have, according to the phase rule.

However, because it can be challenging to graphically express several variables, keeping temperature and pressure constant in the ternary plot's graphical representation is frequently important. This particular version of the phase rule applies to two-component systems under constant pressure. The behavior and compositions of the three-component system under study can be better understood by building and examining the ternary phase diagram. The ternary plot makes complex interactions between components more straightforward to understand by providing a visual depiction.[10]

MATERIALS AND METHODS

Materials

Curcumin was procured from Oxford Lab Fine Chem LLP (Palghar-410210, Maharashtra, India). Clove oil, Cremophor RH ®40, and other excipients were procured from Yarrow Chem products (Mumbai, Maharashtra 400086). A Milli-Q water purification system (ELGA, manufactured in the UK) was used to purify the Milli-Q water. The source of PEG 400 was Sigma Life Science, Sigma Aldrich (Belgium). The supplier of methanol (HPLC grade; 99.9% purity) was Sigma-Aldrich in Steinheim, Germany.

Methods

Screening of the oils and excipients

Studies on the solubility of oils, cosurfactants, and surfactants

To prepare a stable nanoemulsion, excipients such as oil, surfactant, and co-surfactant were selected based on the solubility of curcumin. The oils that were chosen included oleic acid, Peppermint, eucalyptus, lemongrass, castor, anise, clove, coconut, arachis, cinnamon and neem oil. Very few oils were chosen based on their easy accessibility and biodegradability. The chosen surfactants were Tween-20, Cremophor ® RH 40, Tween-80, Polyethylene glycol 200 (PEG 200), Polyethylene Glycol 400 (PEG 400), Span 80 and propylene glycol.

To assess the solubility of curcumin in various components of nanoemulsion, an excess of curcumin was mixed with the chosen solvent (2 mL) in 5 mL stoppered glass vials using vortexing.[11] The glass vials were allowed to acclimate for 48 hr at 25±2°C in a bath shaker. The samples were then subjected to centrifugation for 15 min at rpm of 3000. The supernatant were filtered by a membrane filter. Using a UV-visible spectrophotometer, the supernatant was quantified at 423 nm in order to determine CUR.

Procedure for the construction of ternary phase diagrams

In this study, ternary phase diagrams, which are crucial for the formulation of nanoemulsions, were created using clove oil, the surfactant Cremophor ® RH 40, and the co-surfactant PEG-400. The goal was to determine the ideal surfactant to co-surfactant ratio that would produce an area of nanoemulsification.[12] The solubility analysis led to the optimization of clove oil as an oil phase. For each phase diagram, oil (clove oil) and a particular S_{mix} ratio were completely mixed in volume ratios ranging from 1:9 to 9:1 in separate glass vials. 16 combinations of oil and S_{mix} (1:0.25, 1:0.43, 1:0.66, 1:1, 1:1.5, 1:2, 1:2.33, 1:3, 1:3.5, 1:4, 1:5, 1:6, 1:7, 1:8 and 1:9) were to be identified for the study to be completed. Ternary phase diagrams have been developed by employing the water titration method. Each mixture of oil and S_{mix} was titrated gradually and independently using the aqueous phase. Various aqueous phase volumes were used to obtain a water content of between 5% and 95% of the entire volume at around 5% intervals. At each 5% addition, the percentage of each nanoemulsion component present was calculated to determine the quantity of aqueous phase to be added. For the purpose of a thorough explanation Table 1 provides a comprehensive explanation of oil, surfactant, and cosurfactant in a 1:1 ratio.

Calculation of the percentages of oil, S_{mix}, and water used to build the ternary phase diagram (the ratio of oil to S_{mix} is 1:9).

Using the previous method, 5% of the aqueous phase to the oil:S_{mix} mixture was added and observed, as noted in Table 2. Using visual observation, the following categories were established: Oil/water nanoemulsions that are clear and easily flowable (Figure 1). Clear gel, such as nanoemulsion gel. Emulsion that is milky or hazy (Figure 1). Emulgel, or milky gel.

Selection of the formulation

The nanoemulsion zone of each generated phase diagram was taken into consideration when choosing alternate formulations from the phase diagrams to introduce curcumin into the oil phase.

A dosage of 5 mg of curcumin was chosen to be added to the oil.

For convenience, a 1 mL nanoemulsion formulation was selected, which makes it simple to modify the ratios to meet requirements.

The oil concentration should be such that the drug (single dose) is completely dissolved in it, depending on the drug's solubility in the oil. It is easy to dissolve five milligrams of curcumin (10% of 1 mL) in 0.1 mL of oil.

Different oil concentrations from the nanoemulsion region were chosen at intervals of 5% (10%, 15%, 20%, 25%, etc.) based on each phase diagram.

The effect of curcumin was analyzed with respect to the phase diagram's nanoemulsion area.

The phase diagram was used to determine the lowest concentration of S_{mix} for the preparation of the nanoemulsion for each % of oil chosen.

For the construction of the ternary phase diagram, Chemix school software was used where the data was entered, and it produced the ternary diagram. Based on Figure 3, the desired ratio was selected for the preparation of nanoemulsion.

Preparation of Curcumin loaded nanoemulsion

The spontaneous emulsification method was used in the preparation of Curcumin Nanoemulsion (CNE). The drug was weighed accurately, dissolved in the oil phase, and then sonicated until it was totally soluble. The cosurfactant and surfactant were combined, then water was added dropwise until a uniformly transparent solution was obtained, to prepare the aqueous phase. A nanoemulsion was developed by combining the aqueous and the above oil phase.

Evaluation studies of nanoemulsion

Visual appearance

Oil and surfactant or surfactant mixture was visually inspected by slow addition of water. The samples appear as transparent, translucent, and readily flowable liquid, allowing for their identification as microemulsions. There was no attempt to

Table 1: Calculation of the percentages of oil, S_{mix}, and water used to build the ternary phase diagram (the ratio of oil to S_{mix} is 1:9).

Oil (µL)	S_{mix} (µL)	Water (µL)	Total (µL)	% water (µL)	% S_{mix} (µL)	% oil (µL)
10	90	10	110	9.09	81.82	9.09
10	90	20	120	8.33	75.00	16.67
10	90	25	125	8.00	72.00	20.00
10	90	35	135	7.41	66.67	25.93
10	90	45	145	6.90	62.07	31.03
10	90	55	155	6.45	58.06	35.48
10	90	65	165	6.06	54.55	39.39
10	90	80	180	5.56	50.00	44.44
10	90	100	200	5.00	45.00	50.00
10	90	120	220	4.554	40.91	54.55
10	90	150	250	4.00	36.00	60.00
10	90	185	255	3.51	31.58	64.91
10	90	235	335	2.99	26.87	70.15
10	90	300	400	3.00	23.00	75.00
10	90	400	500	2.00	18.00	80.00
10	90	550	650	2.00	14.00	85.00
10	90	900	1000	1.00	9.00	90.00
10	90	2000	2100	0.48	4.29	95.24

Table 2: Visual observation of different oil and S_{mix} ratio for S_{mix} (1:1). "+" indicates nanoemulsion, "-" indicates emulsion.

Type of emulsion formed after the addition of each aqueous phase																	
Oil: S_{mix} Ratio	10 µL	10 µL	5 µL	10 µL	10 µL	10 µL	10 µL	15 µL	20 µL	20 µL	30 µL	35 µL	50 µL	65 µL	100 µL	150 µL	350 µL
1:9	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
1:4	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
1:2.33	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1:1.5	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:0.66	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:0.43	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:2	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:3	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:5	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-

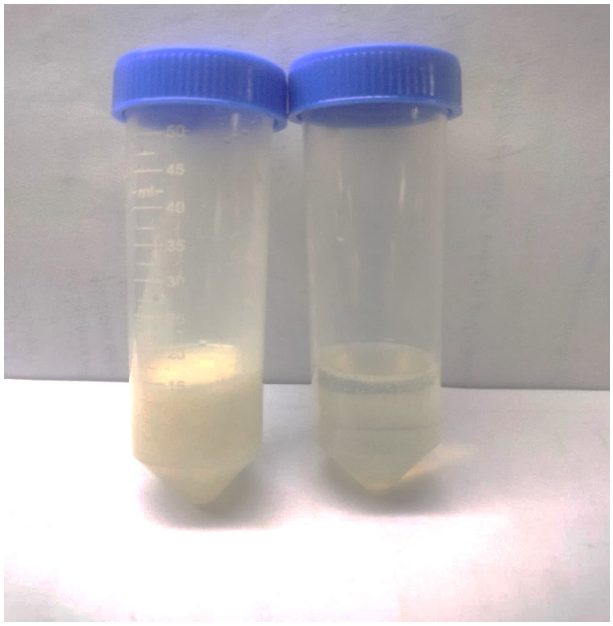

Figure 1: Visual observation of A Emulsion and B Nanoemulsion.

differentiate between the bicontinuous, oil-in-water, and water-in-oil types of emulsions. When the samples appeared as turbid or milky liquids, they were identified as emulsions. When the samples were tilted to a 90-degree angle and the meniscus did not alter, it was determined that they were gel. Using the Chemix School 3.51 program from Arne Standnes USA, all of these categories were plotted as ternary or Pseudoternary phase diagrams on a triangular graph.

Dye-solubility and staining tests

10 µL of a water-soluble dye solution (methylene blue) were added to the nanoemulsion. Provided that the continuous phase in the system is water (o/w emulsion), the dye will dissolve uniformly throughout. The dye will stay as a cluster on the surface of the formulation if the continuous phase is oil.[13]

Measurement of Viscosity

The Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering) was utilized. Using spindle #CPE40 at 25±0.5-C (Laboratories, Inc., Middleboro, MA), the viscosity of the without dilution, formulations (0.5 g) were evaluated (Table 3). The viscosity was computed using Rheocalc V2.6.

Analysis of Droplet Size

The nanoemulsion droplet size was measured using photon correlation spectroscopy as mentioned in Table 3. In a volumetric flask with 50 mL of water, the mixture (0.1 mL) was dispersed, and the flask was tilted slightly to agitate the mixture. The measurement was performed using a Zeta sizer 1000 HS (Malvern Instruments, Worcestershire, UK). Light scattering was seen at a 90° angle at 25°C.

Determination of Zeta Potential

Zeta potential measurements of particle surface charges. Electrophoretic mobility measurements were used to determine the zeta potential using the Horiba Scientific nanoparticle analyzer (nanoPartica SZ-100V2 Series). Immediately after the DS measurements, the potential was measured for each sample using the same cuvette three times in a row. The mean value and standard were then calculated in accordance with Ahmad *et al.*[14-17]

Drug content

The drug content was determined using UV visible spectrophotometer. Ethanol was utilized as a solvent to dilute the formulation, and the absorbance was assessed at a wave length of 423 nm in comparison to a solvent blank. It was determined that the drug content was:

Drug content=Analyzed content/ Theoretical content x 100

……. Equation 1.18

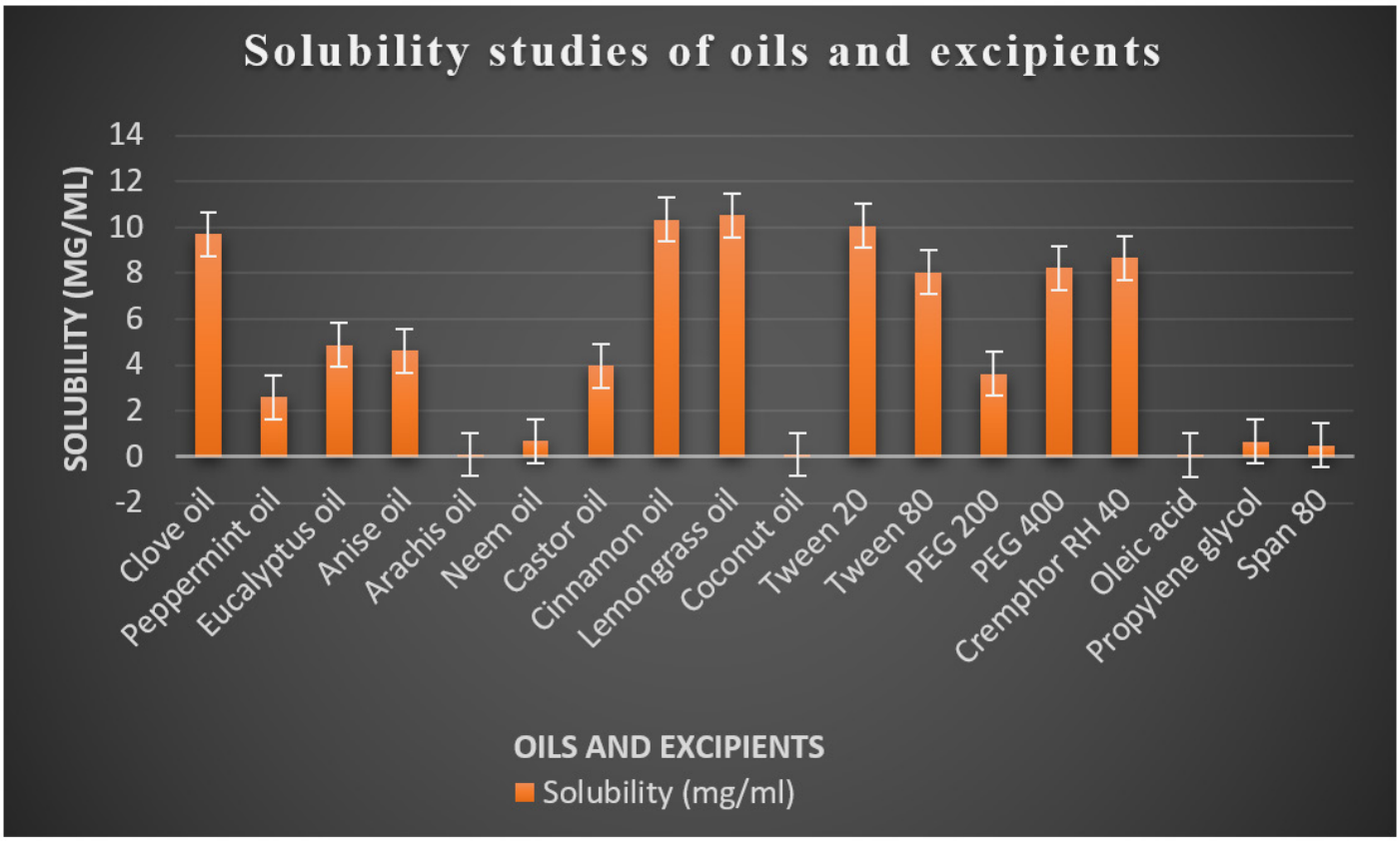

Figure 2: Curcumin's solubility studies in different oils and excipients.

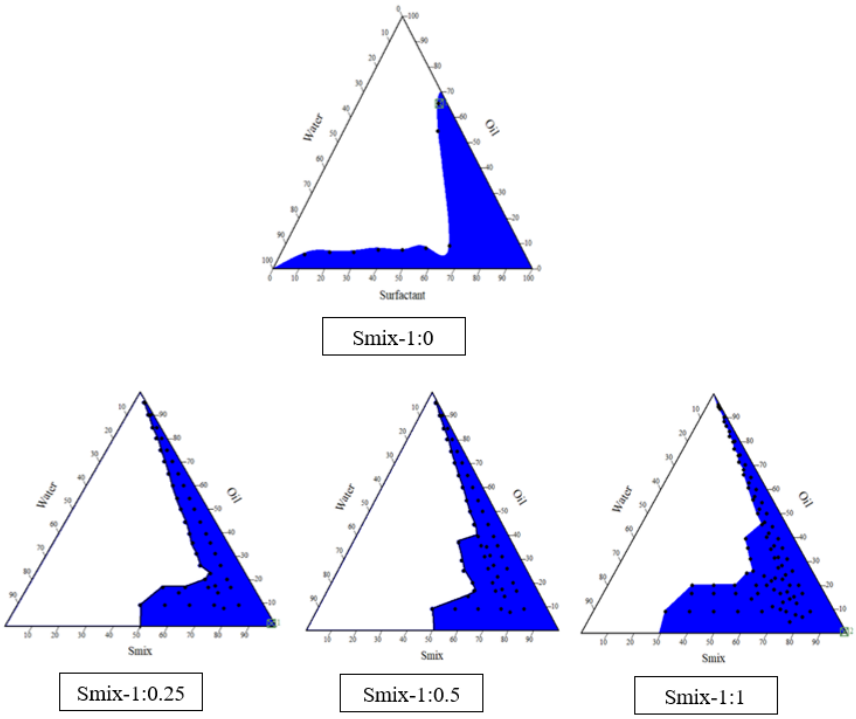

Figure 3: The oil-in-water nanoemulsion (shaded area) region of the ternary phase diagrams for the surfactant Cremophor RH ® 40 and the cosurfactant PEG 400 at different S_{mix} ratios: A (Smix 1:10), B (S_{mix} 1:1), C (S_{mix} 1:2), D (S_{mix} 1:3), E (S_{mix} 1:4), F (S_{mix} 2:1), G (S_{mix} 3:1), and H (S_{mix} 4:1). The regions designated as blue and white, respectively, represent the nanoemulsion and microemulsion zones.

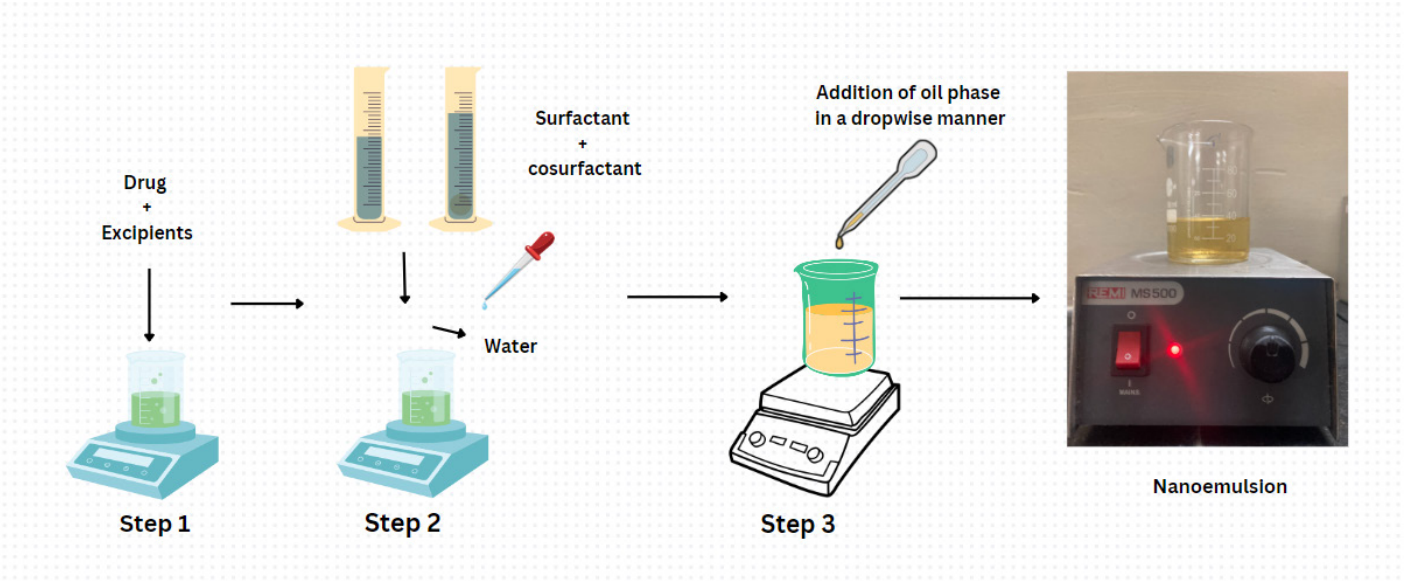

Figure 4: Schematic representation of the method for preparation of curcumin nanoemulsion using the spontaneous emulsification technique.

Table 3: Evaluation studies of curcumin nanoemulsion CUR-CLO NE 1, CUR-CLO NE 2, CUR-CLO NE 3, CUR-CLO NE 4.

Formulation	Particle size	PDI	Zeta potential	Viscosity	Drug content
CUR-CLO NE 1	30.2±0.11	0.145	-38.3±0.23	33±6	98.21±0.12%
CUR-CLO NE 2	40.5±0.36	0.466	-49.0±0.31	39±3	92.34±0.34%
CUR-CLO NE 3	35.4±0.70	1.345	-50.9±0.25	36±4	93.11±0.16%
CUR-CLO NE 4	54.9±0.37	0.285	-47.8±0.22	40±3	90.65±0.23%

(The values are given as mean±S.D., *n*=3).

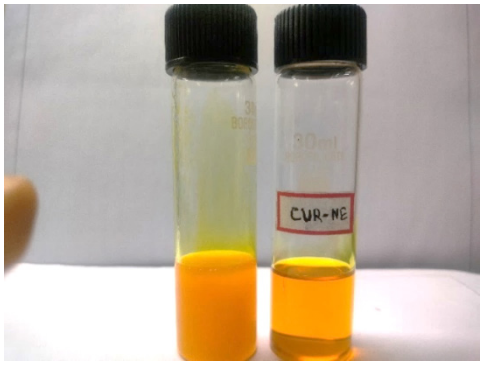

Figure 5: In the above figure, the left side is curcumin microemulsion, and the right shows clear and uniform curcumin nanoemulsion.

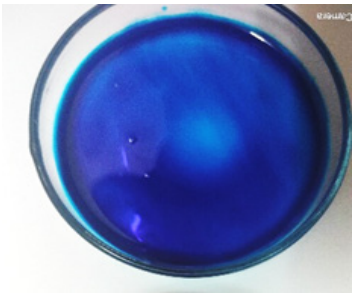

Figure 6: Determination of the type of nanoemulsion using methylene blue dye.

RESULTS

Screening of components

Solubility studies of oils, surfactants and cosurfactants

Curcumin was dissolved in various oils and its solubility was determined. Based on the solubility studies, curcumin showed the highest solubility of 10.52 mg/mL in lemongrass oil, 10.33 mg/mL in cinnamon oil, and 9.7±3.32 mg/mL in clove oil as shown in Figure 2. However, the reason behind selecting clove oil as the oil phase was because of its kinetic stability and its ability to form a stable nanoemulsion, as described by Shahavi *et al*, 2019.[19] Furthermore, according to Alam *et al*[20] its nanoemulsions have a modest propensity for Ostwald ripening. The selection of suitable surfactants and cosurfactants is an important step in the screening and solubility of surfactants and cosurfactants since it is important for the formulation of the nanoemulsion.

For the formation of oil in water (o/w) nanoemulsion, the surfactant's HLB value should always be more than 10. Cremophor RH ® 40 has an HLB value ranging between 12-14.[21] The majority of surfactants utilized in nanoemulsion formulation

Table 4: Viscosity of different curcumin nanoemulsion formulations.

Formulation code	Viscosity
CUR-CLO NE 1	33±6
CUR-CLO NE 2	39±3
CUR-CLO NE 3	36±4
CUR-CLO NE4	40±3

(The values are given as mean±S.D., *n*=3).

are surfactants with single-chain, which may not be able to sufficiently reduce interfacial tension to create nanoemulsions. In these situations, a co-surfactant, or second amphiphile, is added to the solution.[22] For this study, PEG 400, with an HLB value of 11.3, was chosen. Co-surfactants lessen polar head group interactions by intercalating between surfactant molecules. Additionally, they make the interfacial coating around nanoemulsion droplets more flexible.

Ternary phase diagram

A phase diagram can illustrate the connection between a mixture's composition and phase behavior. The o/w NE zones and NE formulation optimization were achieved by the construction of ternary phase diagrams for each S_{mix} ratio (Figure 3). The coloured region indicates the nanoemulsion region in the phase diagrams. The turbid and conventional emulsions are indicated by the white region, based on visual inspection.

From the graph, it is evident that the nanoemulsion region is coloured blue by using the software, and the white region of the graph represents the microemulsion region or the turbid region. When no cosurfactant was added the area of nanoemulsification was low. However, when the cosurfactant was added, the nanoemulsification region increased. Furthermore, the area of nanoemulsification increased when the S_{mix} ratio was increased from 1:0.25 to 1:1. Finally, for the preparation of the nanoemulsion, a 1:1 S_{mix} ratio was chosen.

These findings suggest that the interfacial energy needed for the creation of NE cannot be significantly decreased by the application of a single surfactant. Because of this, a second surfactant, referred to as a co-surfactant, is typically added. This co-surfactant gives the interfacial film enough flexibility to modify its curvature and enable the generation of nanoemulsions over a wide composition range. Moreover, co-surfactants improve the oil's mobility of the hydrocarbon, facilitating improved penetration.

Selection of the formulation

Based on the ternary phase diagram analyses, it was determined that the placebo formulation was adequately stable. As a result, drug-loaded NE was prepared while maintaining the same oil and mix ratios. Enough oil should be used to dissolve the dosage of curcumin in 1 mL of NE. Therefore, 5% oil was selected as the lowest concentration from the ternary diagram. The lowest S_{mix} concentration suitable for the oil concentration, i.e., 10 % surfactant and 10 % cosurfactant in the ratio of 1:1 based on the area of nanoemulsification was chosen. The small nanoemulsion area, which suggests the inability for emulsification for S_{mix} ratios of 1:0, 1:0.25, and 1:0.5 were not used for the formulation of nanoemulsion.

Preparation of Curcumin loaded nanoemulsion

Curcumin nanoemulsions CUR-CLO NE 1, CUR-CLO NE 2, CUR-CLO NE 3, and CUR-CLO NE4 were prepared by spontaneous emulsification method and subjected to further evaluation studies as shown in Figure 4.

Evaluation studies of curcumin nanoemulsion

Visual appearance

The obtained curcumin nanoemulsion was clear, homogenous, and yellow in colour as shown in Figure 5.

Dye-solubility and staining tests

A clear solution was formed when the methylene blue dye was dissolved in 1 µL of the formulation, as seen in Figure 6. The formulation is said to be oil in water (o/w) since there are no color clusters because water is present as a dispersed phase.

Nanoemulsion

Measurement of Viscosity

The viscosity of the formulations CUR-CLO NE 1, CUR-CLO NE 2, CUR-CLO NE 3, and CUR-CLO NE 4 is shown in Table 4. Among all the formulations, CUR-CLO NE 1 has the lowest viscosity. This is due to the presence of low S_{mix} and oil concentration, which is suitable for forming nanoemulsion and its ease of handling.

Analysis of droplet size

Droplet size and PDI are the two important factors for evaluating nanoscale particles and their distribution, with droplet size being a critical factor in the stability and skin penetration of the NE. Droplet size and PDI of CUR-CLO NE 1, CUR-CLO NE 2, CUR-CLO NE 3, and CUR-CLO NE 4 are shown in Figure 7.

The obtained droplet size of CUR-CLO NE 1, CUR-CLO NE 2, CUR-CLO NE 3, and CUR-CLO NE 4 was 30.2±0.11, 40.5±0.36, 35.4±0.70, ands 54.9±0.37 nm with 0.145, 0.466, 1.345, 0.285 PDI respectively as shown in Figure 7. CUR-CLO NE 1 (A) had the smallest droplet size, which was consistently within the NE's size range (10-200 nm), and PDI<0.5 suggested that the size distribution was relevant, but CUR-CLO NE 3 (C) had a PDI value of 1.345 which is >0.5. Hence, it is not acceptable. The investigation by Mohammed S. Algahtani *et al.* (2020) on curcumin nanoemulsion generated by low energy method with

the droplet size range of 10.57 to 68.87 nm and PDI of 0.094 and 0.550 nm supported these findings.

Determination of zeta potential

The amount of surface charge directly affects the stability of NE because surface charges reduce the possibility of coalescence by creating electrostatic interactions between NE globules. The zeta potential of the prepared Curcumin Nanoemulsions (CUR-CLO NE 1, CUR-CLO NE 2, CUR-CLO NE 3, CUR-CLO NE 4) is negative due to the presence of glycol and fatty acids in the anionic groups. Therefore, nanoemulsion formulations with small particle size and negative surface charge can penetrate the skin layers when used in topical formulations as shown in Figure 8.

To determine the overall surface charge and stability of the enhanced Curcumin nanoemulsion formulation, the zeta potential must be determined. The zeta potential values of prepared nanoemulsion formulations were between -38.3±0.25 mV and -50.9±0.23, as shown in Table 3. It was found that the CUR-CLO NE 1 (A) had the lowest zeta potential value, measuring -38.3±0.25 mV. The highest zeta potential value was observed in CUR-CLO NE 3 (C), with a measurement of -50.0±0.27 mV. Zeta potential values that are negative may result from the presence of negatively charged fatty acid esters in clove oil.[23,24]

DISCUSSION

The solubility of a drug in excipients must be considered while selecting the components of the NE formula, especially for the oil phase. Drugs that are not very water soluble, like CUR, will mostly diffuse in the oil phase. Consequently, the NE's ability to load drugs will be enhanced by an oil phase that can dissolve CUR effectively. In addition, choosing appropriate surfactants and co-surfactants for the formula depends critically on assessing the emulsification ability. This is a crucial test to make sure nanoemulsion satisfies the prerequisites for uniformity and clarity, particularly for NEs made with low-energy techniques. However, the construction of a phase diagram provides a method for roughly estimating the relationship between the ratios of the

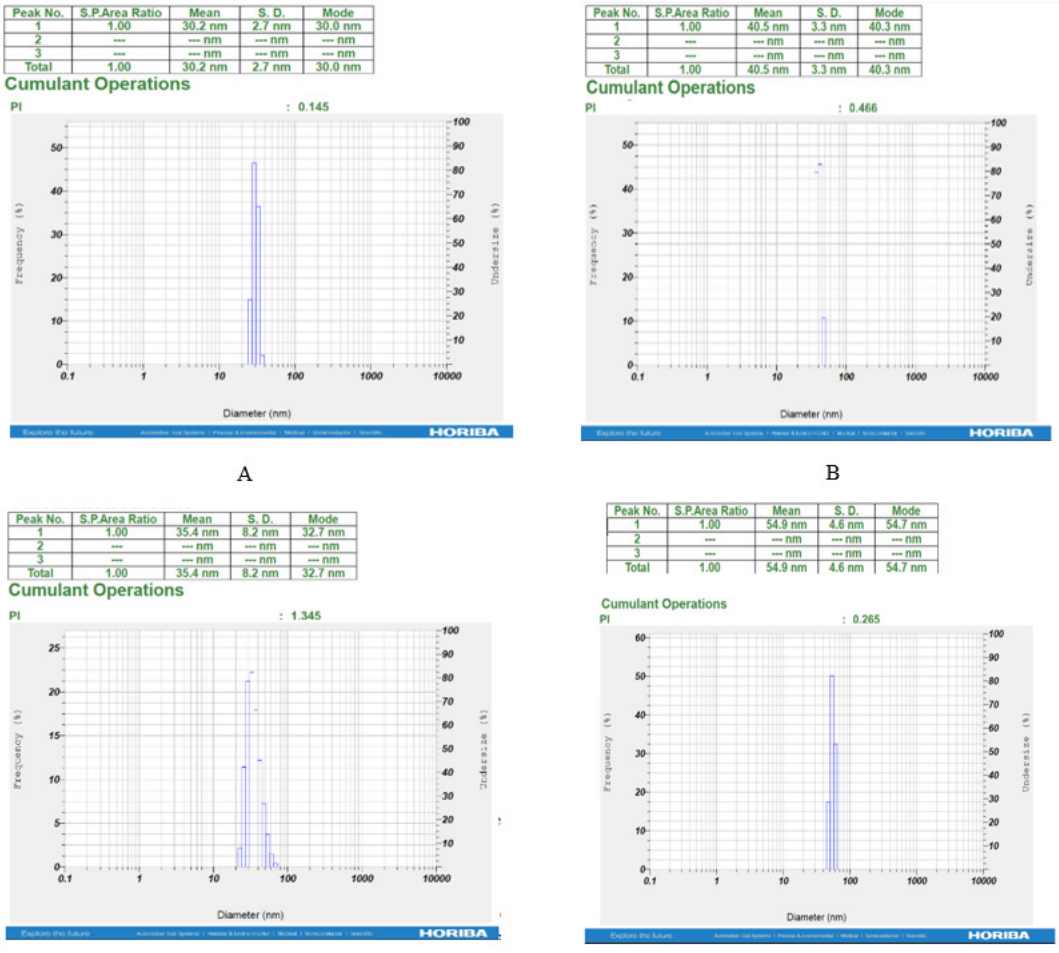

Figure 7: Particle size distribution of CUR-CLO NE 1 (A) and CUR-CLO NE 2 (B). CUR-CLO NE 3 (C), and CUR-CLO NE 4 (D) using dynamic light scattering technique.

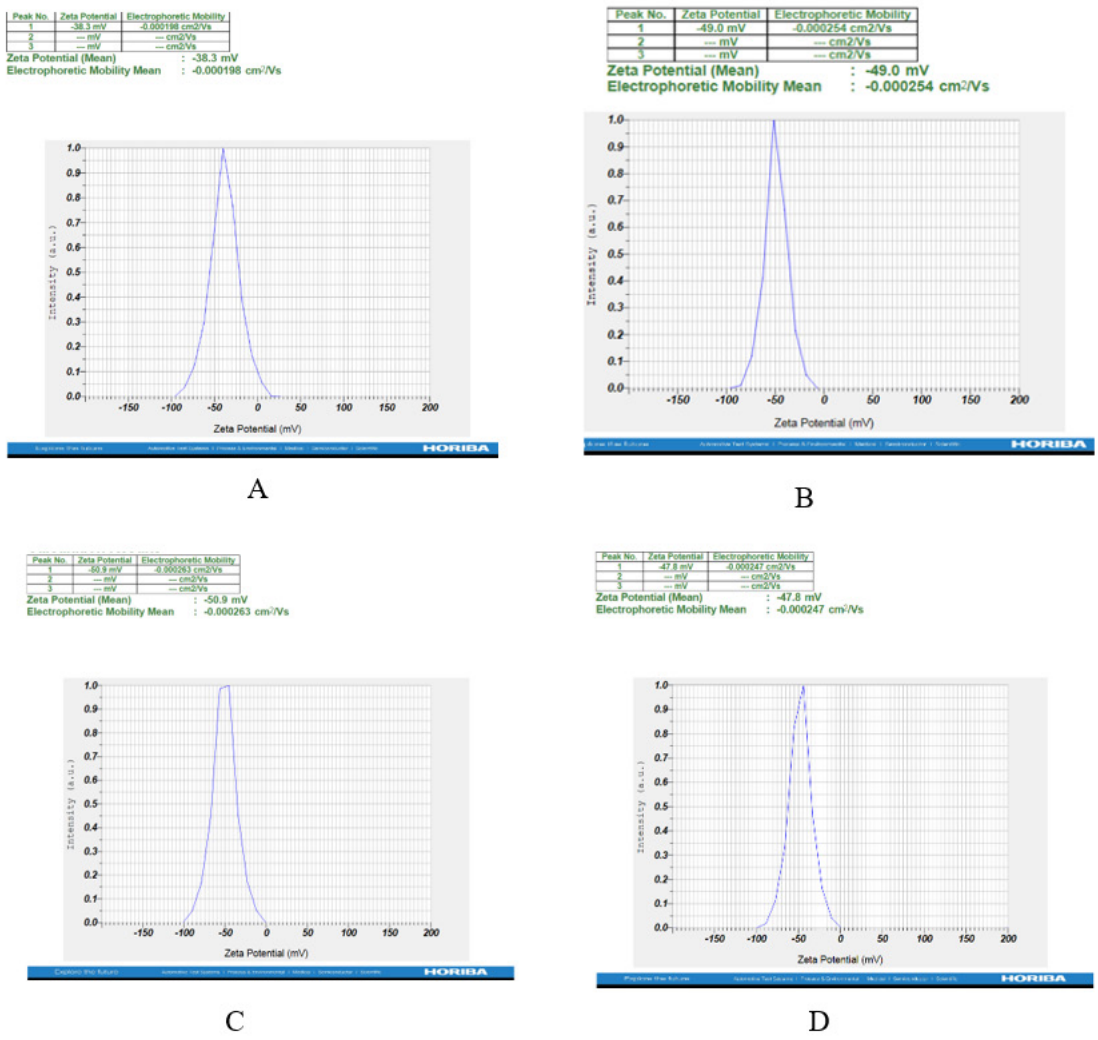

Figure 8: Determination of zeta potential of curcumin nanoemulsion (A) CUR-CLO NE 1 (B) CUR-CLO NE 2 (C) CUR-CLO NE 3 (D) CUR-CLO NE 4.

three main components of the formula—oil, S_{mix}, and water-that come together to deliver the mixture.

Ternary phase study aids in identifying the proper ratio for the three components, which is the area on the phase diagram that produces the emulsion. Two fundamental metrics for assessing nanoscale particles and their distribution are droplet size and PDI, respectively. Droplet size is crucial for the nanoemulsion's stability and skin penetrating capabilities. The obtained droplet size was consistently within the NE's size range of 10-100 nm, and PDI<0.5. These conclusions were supported by Ahmad *et al.*'s (2019) study on CUR-NE made by ultrasonication with a droplet size range of 50.85 to 188.60 nm and a PDI of 0.256 to 0.55918.

In terms of the influence of the formulation ingredients on droplet size, the study found that the droplet size was much greater in the regions with the lowest water content and the highest oil content, as well as when the water ratio was high and the amount of oil was low as shown in Figure 3 depicting the ternary phase diagram of different S_{mix} ratios. Furthermore, the studies showed that the interaction between these variables reduced the droplet size of CUR-CLO NE 1, which was due to greater S_{mix} and lower oil levels. With respect to the impact of S_{mix} and water ratios on PDI, results showed that excessive surfactant molecules in the Smix ratio could result in the formation of aggregates or micelles, causing an increase in the PDI in the case of Figure 7 (C) CUR-CLO NE 3.

The prepared curcumin nanoemulsion fulfilled the requirements suitable for an oil-in-water nanoemulsion via the dye test. It has a clear appearance without any particles. Therefore, the oil and excipients used in this study, including clove oil, polyethylene glycol 400, and Cremophor RH ®40, were proven successful in the formulation of curcumin nanoemulsion.

CONCLUSION

This current research has unequivocally demonstrated that different curcumin nanoemulsions have been developed by using spontaneous emulsification techniques as proven by ternary phase diagrams. The formulation's stability and solubility studies led to the selection of clove oil as the oil phase. Later, the selection of the

surfactants and cosurfactants and the optimized ratio were done based on the ternary phase diagram. A S_{mix} ratio of 1:1 showed the highest nanoemulsification region, which was further used to prepare stable nanoemulsions. This ternary phase diagram explains the main criteria for selecting formulations with the lowest surfactant concentration in the shortest amount of time from the phase diagrams, avoiding metastable formulations. Hence, the optimized formulation contained 15 % clove oil, 20 % S_{mix}, and 10 % water, resulting in the preparation of a stable nanoemulsion. Further evaluation studies reported that the optimized nanoemulsion formulation CUR-CLO NE 1 attained an appropriate droplet size of 30.2±0.11 nm, which had the lowest droplet size compared to other curcumin nanoemulsion formulations CUR-CLO NE 2, CUR-CLO NE 3, and CUR-CLO NE 4 when determined by particle size analyzer. The zeta potential value of CUR-CLO NE 1 was 38.3±0.25 mV which determined its capability of penetrating into the skin layers via topical application. The most crucial evaluation parameter was the drug content estimation, which revealed that 91.21% of the medication was entrapped in the formulation. The above investigation reported that poorly soluble drugs like curcumin can be delivered using nanoemulsion and was proven successful in the formulation of a stable nanoemulsion.

ACKNOWLEDGEMENT

The authors acknowledge UGC for providing funding under the Savitribai Jyotirao Phule Single Girl Child Fellowship (SJSGC) under Grant Number F. No. 82-7/2022(SA-III) and to GITAM School of Pharmacy (Visakhapatnam) for providing all the necessary facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CUR: Curcumin; **S_{mix}:** Surfactant and cosurfactant mixture; **PEG 400:** Polyethylene glycol 400; **CUR-CLO NE 1:** Curcumin nanoemulsion 1; **CUR-CLO NE 2:** Curcumin nanoemulsion 2; **CUR-CLO NE 3:** Curcumin nanoemulsion 3; **CUR-CLO NE 4:** Curcumin nanoemulsion 4; **US FDA:** United States Food and Drug Administration **NE:** Nanoemulsion; **PDI:** Polydispersibility index.

REFERENCES

1. Alsarra IA, Bosela AA, Ahmed SM, Mahrous GM. Proniosomes as a drug carrier for transdermal delivery of ketorolac. Eur J Pharm Biopharm. 2005;59(3):485-90. doi: 10.1016/j.ejpb.2004.09.006, PMID 15760729.
2. Thakur R, Anwer MK, Shams MS, Ali A, Khar RK, Shakeel F, *et al.* Proniosomal transdermal therapeutic system of losartan potassium: development and pharmacokinetic evaluation. J Drug Target. 2009;17(6):442-9. doi: 10.1080/10611860902963039, PMID 19527115.
3. Gigliobianco MR, Casadidio C, Censi R, Di Martino P. Nanocrystals of poorly soluble drugs: drug bioavailability and physicochemical stability. Pharmaceutics. 2018;10(3):134. doi: 10.3390/pharmaceutics10030134, PMID 30134537.
4. Liu P, Chen G, Zhang J. A review of liposomes as a drug delivery system: current status of approved products, regulatory environments, and future perspectives. Molecules. 2022;27(4):1372. doi: 10.3390/molecules27041372, PMID 35209162.
5. Khan S, Sharma A, Jain V. An overview of nanostructured lipid carriers and its application in drug delivery through different routes. Adv Pharm Bull. 2023;13(3):446-60. doi: 10.34172/apb.2023.056, PMID 37646052.
6. Chauhan I, Yasir M, Verma M, Singh AP. Nanostructured lipid carriers: A groundbreaking approach for transdermal drug delivery. Adv Pharm Bull. 2020;10(2):150-65. doi: 10.34172/apb.2020.021, PMID 32373485.
7. Sari TP, Mann B, Kumar R, Singh RR, Sharma R, Bhardwaj M, *et al.* Preparation and characterization of nanoemulsion encapsulating curcumin. Food Hydrocoll. 2015;43:540-6. doi: 10.1016/j.foodhyd.2014.07.011.
8. Int J Intell Eng Syst. 2022;15(4).
9. Syed K, Peh KK. Identification of phases of various oil, surfactant/co-surfactants and water system by ternary phase diagram. Acta Pol Pharm. 2014;71:301-9.
10. Ahmad J, Amin S, Kohli K, Mir SR. Construction of pseudoternary phase diagram and its evaluation: development of self-dispersible oral formulation. IT Medical Team.
11. Algahtani MS, Ahmad MZ, Ahmad J. Nanoemulsion loaded polymeric hydrogel for topical delivery of curcumin in psoriasis. J Drug Deliv Sci Technol. 2020;59:(101847). doi: 10.1016/j.jddst.2020.101847.
12. Le TT, Nguyen TK, Nguyen VM, Dao TC, Nguyen HB, Dang CT, *et al.* Development and characterization of a hydrogel containing curcumin-loaded nanoemulsion for enhanced *in vitro* antibacteria and *in vivo* wound healing. Molecules. 2023;28(17):6433. doi: 10.3390/molecules28176433, PMID 37687262.
13. Feng SM, Zhao Y, Xu Q, Li HM, Huang YX, Liu HH, *et al.* Development and characterization of A new dimethicone nanoemulsion and its application for electronic gastroscopy examination. Int J Nanomedicine. 2020;15:5405-16. doi: 10.2147/IJN.S251113, PMID 32801696.
14. Ahmad N, Alam MA, Ahmad FJ, Sarafroz M, Ansari K, Sharma S, *et al.* Ultrasonication techniques used for the preparation of novel eugenol-nanoemulsion in the treatment of wounds healings and anti-inflammatory. J Drug Deliv Sci Technol. 2018;46:461-73. doi: 10.1016/j.jddst.2018.06.003.
15. Ahmad N, Ahmad R, Alam MA, Ahmad FJ, Amir M. Impact of ultrasonication techniques on the preparation of novel amiloride-nanoemulsion used for intranasal delivery in the treatment of epilepsy. Artif Cells Nanomed Biotechnol. 2018;46;sup3:S192-207. doi: 10.1080/21691401.2018.1489826, PMID 30032652.
16. Ahmad N, Alam MA, Ahmad FJ, Sarafroz M, Ansari K, Sharma S, *et al.* Ultrasonication techniques used for the preparation of novel eugenol-nanoemulsion in the treatment of wounds healings and anti-inflammatory. J Drug Deliv Sci Technol. 2018;46:461-73. doi: 10.1016/j.jddst.2018.06.003.
17. Ahmad N, Ahmad R, Naqvi AA, Alam MA, Ashafaq M, Abdur Rub R, *et al.* Intranasal delivery of quercetin-loaded mucoadhesive nanoemulsion for treatment of cerebral ischemia. Artif Cells Nanomed Biotechnol. 2018;46(4):717-29. doi: 10.1080/21691401.2017.1337024, PMID 28604104.
18. Ahmad N, Ahmad R, Al-Qudaihi A, Alaseel SE, Fita IZ, Khalid MS, *et al.* Preparation of a novel curcumin nanoemulsion by ultrasonication and its comparative effects in wound healing and the treatment of inflammation. RSC Adv. 2019, January 1;9(35):20192-206. doi: 10.1039/c9ra03102b, PMID 35514703.
19. Shahavi MH, Hosseini M, Jahanshahi M, Meyer RL, Darzi GN. Evaluation of critical parameters for preparation of stable clove oil nanoemulsion. Arab J Chem. 2019;12(8):3225-30. doi: 10.1016/j.arabjc.2015.08.024.
20. Alam P, Ansari MJ, Anwer MK, Raish M, Kamal YK, Shakeel F. Wound healing effects of nanoemulsion containing clove essential oil. Artif Cells Nanomed Biotechnol. 2017;45(3):591-7. doi: 10.3109/21691401.2016.1163716, PMID 28211300.
21. Tang H, Xiang S, Li X, Zhou J, Kuang C. Preparation and *in vitro* performance evaluation of resveratrol for oral self-microemulsion. PLOS ONE. 2019;14(4):e0214544. doi: 10.1371/journal.pone.0214544, PMID 30990813.
22. British Pharmacopoeia. XXX. London: Medicines and Healthcare products Regulatory Agency; 2016. p. 741.
23. Bali V, Ali M, Ali J. Study of surfactant combinations and development of a novel nanoemulsion for minimising variations in bioavailability of ezetimibe. Colloids Surf B Biointerfaces. 2010;76(2):410-20. doi: 10.1016/j.colsurfb.2009.11.021, PMID 20042320.
24. Alam P, Ansari MJ, Anwer MK, Raish M, Kamal YK, Shakeel F. Wound healing effects of nanoemulsion containing clove essential oil. Artif Cells Nanomed Biotechnol. 2017;45(3):591-7. doi: 10.3109/21691401.2016.1163716, PMID 28211300.

Cite this article: Joshna B, Sirisolla JD. Spontaneous Emulsification as a Novel Approach for the Preparation and Characterization of Curcumin Nanoemulsions: Advancing Bioavailability and Therapeutic Efficacy. J Young Pharm. 2025;17(1):166-75.