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Formulation Optimization and Evaluation of Nanoemulgel Loaded with Plant Extract of Crinum Latifolium for Wound Healing Potential

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Abstract

Crinum Latifolium (CL) is a well-known plant known for its anti-oxidant, anti-inflammatory and anti-arthritic activity belonging to family Amaryllidaceae. Leaves part of that has tremendous anti-arthritic activity. The objective of this study was to formulate a topical nanoemulgel formulation from methanolic extract of Crinum Latifolium for the treatment of rheumatoid arthritis (RA) with the intention of minimizing the systemic side effects. First, the methanolic extract of Crinum Latifolium (MCL) was obtained by Maceration method. The nanoemulsion formulation of Crinum Latifolium methanolic extract (NE-MCL) was prepared through the hot emulsification method followed by homogenization technique. The optimization of surfactant and co-surfactant concentration was carried out using phase diagrams method and found that is able to formulate a stable emulsion. This ratio was further investigated for the process parameters i.e. Oil: Smix (X1), stirring speed (X2), and stirring time (X3). The final optimized formulation was found to have particle size (PS) of 225 nm and polydispersity index of 0.128 and zeta potential of -3.198mV with a desirability of 0.846. The optimized formulation was consisted of 98.33 ± 0.69% drug content. *In vitro* drug release studies showed that about 80% of the MCL was released in 24 hrs from the NE-MCL represented its sustained release effect. The TEM micrograph showed a perfectly spherical shaped particles in nano size range. The NE-MCL gel (NEG-MCL) was formed using Carbopol ETD 2020 and the pH was measured. The pH was found to have a value of 5.78 ± 0.12, ensuring that no bacterial growth or tissue irritation occurs due to pH fluctuations. The spreadability and viscosity of the nanoemulgel formulation was found to be 22.84 cm and 247.10 ± 1.3 cps respectively. The *in vitro* drug release of nanoemulgel formulation was compared to plain Nanoemulsion formulation and found that Nanoemulgel showed a better sustained release effect compared to plain Nanoemulsion formulation. Also, the R^2 value for zero order, first order, Higuchi model and Koresmeyer Peppas model was found to be 0.782, 0.815, 0.912 and 0.892 respectively which showed that the drug was release by diffusion-controlled mechanism from the matrix. These results showed that nanoemulgel formulation is capable to provide a sustained release formulation which in turn helped in reducing the dosage and dosing frequency and thereby, reduces the adverse effects of MCL.

Keywords: Optimization, Phase Diagrams, Nanoemulgel, Topical delivery, Rheumatoid arthritis

Introduction: -

Rheumatoid arthritis is well known worldwide for its chronic inflammatory autoimmune condition that triggers inflammation within the joints, leading to degradation of cartilage and loss of bone tissue. While a definitive cure for RA remains elusive, the past two decades have witnessed the emergence of medications that slows down the disease progression and deter joint deformities, constituting a prolonged management strategy [1]. Recent studies have revealed the increased therapeutic effectiveness of first-generation tyrosine kinase inhibitors (TKIs), but fails to achieve the desired therapeutic outcomes with minimum adverse effects. The present study is dedicated to exploring the potential in treating rheumatoid arthritis through a topical delivery system [2].

Ayurveda is one of the greatest gifts of the sages which ancient India has given to mankind. More than 70% of India's populations still use these non-allopathic systems of medicine. Existing synthetic drugs for the treatment of arthritis have several limitations due to extra-articular manifestations or co -morbidities. The modern medicine confesses that ayurveda and herbal medicine, has a lot of positive influence in the treatment of arthritis. Many herbal drugs/agents either in crude form or isolated component could be a better alternative in the treatment of arthritis [3].

Crinum Latifolium is a plant part (leaves) with genus Crinum belonging to family Amaryllidaceae. It belongs to a sub-family i.e. Amaryllidoideae. The tribe and subtribe for the plant was found to be Amaryllideae and Crininae respectively. The Crinum Latifolium was found to have various pharmaceutical activities: antimicrobial activity, anti – inflammatory activity, antitumor activity, anthelmintic activity, antibacterial and anticancer activity [4,5]. So, in this research work, we investigated this plant for its anti-arthritic and anti-inflammatory activity.

The aim of this research was formation of nanoemulgel of methanolic extract of leaves of Crinum Latifolium to increase its therapeutic activity and reduce its side-effects. First, the solubility of the plant extract was investigated in various oils for maximum drug solubility. Second, the nanoemulsion was optimized for the surfactants-cosurfactant ratio and different

process parameters for minimum globule size and maximum drug release. The final optimized nanoemulsion was incorporated into the gel and evaluated for various evaluation parameters.

1. Methods and materials

a. Materials

Peceol, geleol, labrosol, caproyl 90, and transcutol-P were generously provided as a kind sample by Gattefosse (Mumbai, India). Dialysis membranes (molecular weight cut-off of 12,000–14,000) and membrane filters (0.22 μm) were procured from Merck (India). All remaining laboratory reagents were acquired from HiMedia (Mumbai, India).

b. Formation of Optimized Nanoemulsion Formulation

The nanoemulsion formulation was prepared by emulsification followed by homogenization method [6]. The solubility of the drug signifies the amount of drug that are to be incorporated into oil phase of the NE formulation. First, a precise quantity of drug was introduced into the oily phase and subjected to sonication at 60°C until complete dissolution of the drug was achieved. Second, the aqueous phase was formulated by dissolving a known concentration (2%) of surfactant into water. Add this aqueous surfactant phase drop by drop to oily phase and stirred it vigorously under continuous stirring using mechanical stirrer until a clear NE was obtained. The resultant nano-emulsion underwent homogenization at 10,000 rpm for 10 minutes and was subsequently cooled to room temperature [7].

c. Preliminary Formulation Studies

i. Solubility Analysis in Various Solvents

The solubility of MCF was assessed in different oils i.e. Cinnamon oil, Lavender oil, Long oil, Anise oil, Rosemary oil, Garlic oil, Turmeric oil, Ginger oil, Sunflower oil, Lemon grass oil, Kalongi oil, Eucalyptus oil, Coriander oil and surfactants, including peceol, span 80, capryol, transcutol, tween 80, labrafil, and labrafac to identify the maximum solubility. The oils and surfactants were placed into small glass vials. An excess amount of the drug was added to each vial. These vials were tightly sealed and subjected to continuous stirring for 72 hours at 25°C on a mechanical shaker (REMI, India) until reaching equilibrium. Following equilibration, the samples were centrifuged at 12,000 rpm for 10 minutes using a cooling centrifuge (REMI Pvt. Ltd., Mumbai, India). The resulting supernatant was then separated and the solubility was determined using UV spectroscopy after appropriate dilution with methanol. All measurements were conducted in triplicate, and the average values were utilized for analysis [8].

ii. Interpretation of Pseudo ternary Phase Diagrams

The ratio of the surfactant and co-surfactant used in the nano emulsion formulation were optimized using pseudo ternary phase diagrams by employing the spontaneous emulsification method [9, 10]. The blend of surfactant and cosurfactant (Smix) are prepared in predetermined weight ratios (1:1, 1:2, 1:3, 2:1, 3:1, and 4:1). Portions of each surfactant and cosurfactant blend were then combined with oil at room temperature in the given weight ratios as follows 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 (w/w). Water was gradually introduced into each oil–Smix blend under continuous stirring. The visual examination was carried out after attaining the equilibrium. The preparation process did not involve any heating. The phase diagrams were generated using CHEMIX 3.51 software (MN, USA). The composition of mixtures at different points in the phase diagrams was expressed as %A (Oil) + %B (Smix) + %C (Water) = 100.

iii. Design of Experiment

Apart from concentration of oil, surfactant, and co-surfactant, various process parameters, stirring speed, and stirring time also significantly affect the therapeutic efficacy and stability of the formulation. To address this, a 2^3 full factorial design, was implemented to evaluate the effect of the independent variables i.e. Oil: Smix (X1), stirring speed (X2), and stirring time (X3) on the response variables i.e. globule size and *in vitro* drug release.. An optimistic approach was employed to evaluate the linear, quadratic, and interaction effects of independent factors on the dependent variables [11]. Design Expert software (version 8.07) was employed a total of 8 experiments. Table 1.

Table 1: Independent and dependent variables for 2^3 full factorial design

Formulation No.	Coded value			Actual values		
	X1	X2	X3	Oil: Smix	Stirring Speed (rpm)	Stirring time (Min.)
1	1	1	-1	60	1200	15
2	1	1	1	60	1200	20
3	1	-1	1	60	1000	20
4	1	-1	-1	60	1000	15
5	-1	1	-1	40	1200	15

6	-1	-1	-1	40	1000	15
7	-1	1	1	40	1200	20
8	-1	-1	1	40	1000	20

iv. Optimization, Data Analysis & Desirability Function

Formulations were optimized through Design expert software. Polynomial models including linear and quadratic equations were generated for all the response variables. The significance and confidence limit of the designed experiment were statistically estimated via analysis of variance (ANOVA) as depicted in Table **2**. After employing this hypothetical testing, the desirability function was used for the precision and accuracy of the experiments. This approach unites all the responses over one variable to predict optimistic independent variables [12]. Optimized batch was further explored for characterization studies

d. Characterization of Nanoemulsion Formulation

i. Zeta Potential (ZP), Particle Size (PS), and Polydispersity Index (PDI) Measurement

These parameters were determined using the dynamic light scattering (DLS) technique on a Malvern Zeta Sizer (Nano ZS, Malvern Instruments, UK) with a 90° angle of detection at 25°C. For analysis, approximately 100 µL of the emulsion was taken, diluted to 1 mL (1:10 dilution), and then assessed for size, PDI, and ZP [13].

ii. Drug Content (%DC)

The percent drug content was assessed by isolating the free drug within the nano emulsions through the dialysis method. In this process, 1 g of MCL-loaded emulsion was placed in a dialysis tube with a molecular weight cut-off (MWCO) of 3.5 kDa, and both ends were securely sealed. The sealed dialysis bag was immersed in 250 mL of distilled water at 37 ± 0.5 ◦C and subjected to stirring at 200 rpm. After one hour, 5 mL of the sample was withdrawn and examined by UV spectrophotometer [14].

iii. Study of *In vitro* Drug Release

The *in vitro* drug release study was conducted using the dialysis bag method, involving optimized emulsion and free drug solutions [15]. A cellulose membrane with a molecular weight cutoff of 10,000 Da was employed and underwent an overnight soaking period for

equilibration in PBS, pH 6.4. The release medium consisted of phosphate buffer at pH 5.5, supplemented with 1% triton X100, and the temperature was maintained at 37 ± 0.5 ◦C for a duration of 24 hours, with continuous stirring at 400 rpm. 1 mL samples were withdrawn at predetermined time points and replaced with an equal volume of fresh medium to ensure sink conditions. Subsequently, the samples were suitably diluted and analyzed spectrophotometrically. The drug release kinetics and mechanisms of the optimized formulation were assessed using different kinetic models. The selection of the best-fit model was based on the R^2 values obtained from each of the models [16].

iv. Transmission Electron Microscopy

The morphological characteristics (shape and surface) of the optimized batch were observed by high resolution transmission electron microscope (Morgagni 268 D). The sample was diluted 1000 times, applied to a carbon coated 300-mesh and the photomicrograph were taken [17].

e. Method of Preparation of Nanoemulgel (NEG-MCL)

The nanoemulgel formulation was prepared by employing the optimized nanoemulsion formulation to the aqueous phase of the gelling agent. For this, 0.5% Carbopol ETD 2020 was added to the remaining 60% Milli-Q water, and the mixture was neutralized to pH 5.5 using a 1% NaOH solution, forming a gel at room temperature under continuous homogenization. The prepared nanoemulsion formulation was then added dropwise to the gel base with continuous stirring at room temperature, resulting in the creation of the MCL loaded nano-emulsion-gel [18].

f. Characterization of Nanoemulgel Formulation

i. Determination of pH

The pH of the prepared emulgel was determined using a calibrated handheld pH meter. A 10% dispersion of the emulgel was prepared with Milli-Q water, and the pH measurement was recorded at room temperature [19].

ii. Spreadability Analysis

The evaluation of the spreadability of the optimized emulgel was conducted through the graph paper method. Two pre-weighed glass slides were positioned over graph paper, displaying the

x and y axes, with a square outlined on the chart paper. A quantity of 0.5 g of the gel was applied to the glass slide within the designated square. Subsequently, the second glass slide was placed over the first, and weights were added progressively. The incremental diameter of the spread gel was then recorded [20].

iii. Determination of Viscosity

The viscosity of the chosen nanoemulgel formulation was assessed with an Anton Paar MCR 92, employing a flat-faced spindle with a consistent shear rate of 10 at 25 ◦C [21]. The average viscosity was determined based on twenty data points.

iv. Evaluation of drug Content

The effectiveness of any formulation relies significantly on its drug content. For the quantification of MCF content, 2 ml of the nanoemulsiongel, equivalent to 25 mg of the drug, was placed in a volumetric flask and diluted to a total volume of 10 ml with PBS at a pH of 6.4. The resulting solution underwent sonication for a duration of 10 minutes and was subsequently filtered using a 0.45 µm membrane filter. After appropriate dilution, the drug content was analyzed spectrophotometrically for percentage drug content. This procedure was iterated three times, and the average value derived from these three repetitions was employed to establish the assay [22].

v. *In vitro* release studies and release kinetics

The *in-vitro* drug release testing of the nanoemulgel was conducted within a Franz diffusion cell using phosphate-buffered saline (PBS, pH 6.4). This activated cellulose membrane was positioned between the donor and receptor compartments of the Franz diffusion cell, featuring an effective diffusion area of 2.26 cm^2 and a cell volume of 25 ml. The receptor compartment was charged with PBS pH of 6.4. The Franz diffusion cells were consistently maintained at a temperature of 37°C, employing magnetic stirring at a constant speed of 50 rpm for a duration of 24 hours. A 2 ml formulation, equivalent to 25 mg of the drug, was delicately introduced into the donor compartment. Samples were periodically withdrawn over the course of 24 hours. 1 ml aliquots were withdrawn from the dissolution medium and substituted with fresh PBS at predefined time intervals. The samples were then subjected to spectrophotometric analysis to determine the drug content [23]. The drug release kinetics and mechanisms of the optimized formulation were also evaluated as discussed earlier. The selection of the best fit was determined by evaluating the R^2 values obtained from each of the models.

g. Investigations of Thermodynamic Stability

The optimized nanoemulgel underwent various thermodynamic stability assessments, which included a centrifugation test, freeze-thaw study, and heat-cooling cycle. In the centrifugation test, 1 g of the optimized nanoemulsion was mixed with water to achieve a total volume of 10 mL and then subjected to centrifugation at 10,000 rpm for 10 minutes using a REMI CPR-24 centrifuge. The emulsion was subsequently inspected for any signs of instability. For the freeze-thaw stability assessment, the emulsion underwent cycles of freezing at -21°C for 24 hours followed by thawing at +25°C for three cycles. Additionally, the formulation was exposed to three heating-cooling cycles, with the cooling phase set at 4°C and the heating phase at 45°C for over 48 hours, adhering to the specified parameters for the heating cycle [24].

2. Results and Discussion

a. Preliminary Formulation Studies

i.Solubility Analysis in solvents

The solubility of MCF was investigated in different oils (Table 2) and found to be maximum in cinnamon oil. The solubility of was also investigated in various surfactants and found to be maximum in Tween 80 and Span 80. Thus, used as a surfactant and co-surfactant in the formulation of Nanoemulsion.

Table 2: Solubility Analysis in Various solvents

S.no.	Oil	Solubility
1.	Lavender oil	Insoluble
2.	Long oil	Slightly soluble
3.	Anise oil	Partially soluble
4.	Rosemary oil	Insoluble
5.	Garlic oil	Partially soluble
6.	Turmeric oil	Insoluble
7.	Cinnamon oil	Soluble
8.	Ginger oil	Slightly soluble
9.	Sunflower oil	Insoluble
10.	Lemon grass oil	Partially soluble
11.	Kalongi oil	Insoluble
12.	Eucalyptus oil	Partially soluble
13.	Coriander oil	Insoluble

Table 3: Solubility analysis in various surfactants

S.no.	Surfactant	Solubility
1.	Peceol	Insoluble
2.	Span 80	Soluble
3.	Capryol	Insoluble
4.	Transcutosol	Insoluble
5.	Tween 80	Soluble
6.	Labrafil	Insoluble
7.	Labrafac	Insoluble

ii. Interpretation of Pseudoternary Phase Diagrams

These diagrams were constructed using the spontaneous emulsification method with fixed weight ratios (1:1, 1:2, 1:3, 2:1, 3:1, and 4:1) using CHEMIX 3.51 software (MN, USA) as illustrated in Figure 1. The composition of mixtures at various points was determined by the expression %A (Cinnamon oil) + %B (Tween 80: Span 80) + %C (Water) = 100. The largest nanoemulsion region was observed for the surfactant: co-surfactant ratio of 1:1, indicating its capability to produce stable emulsions [25].

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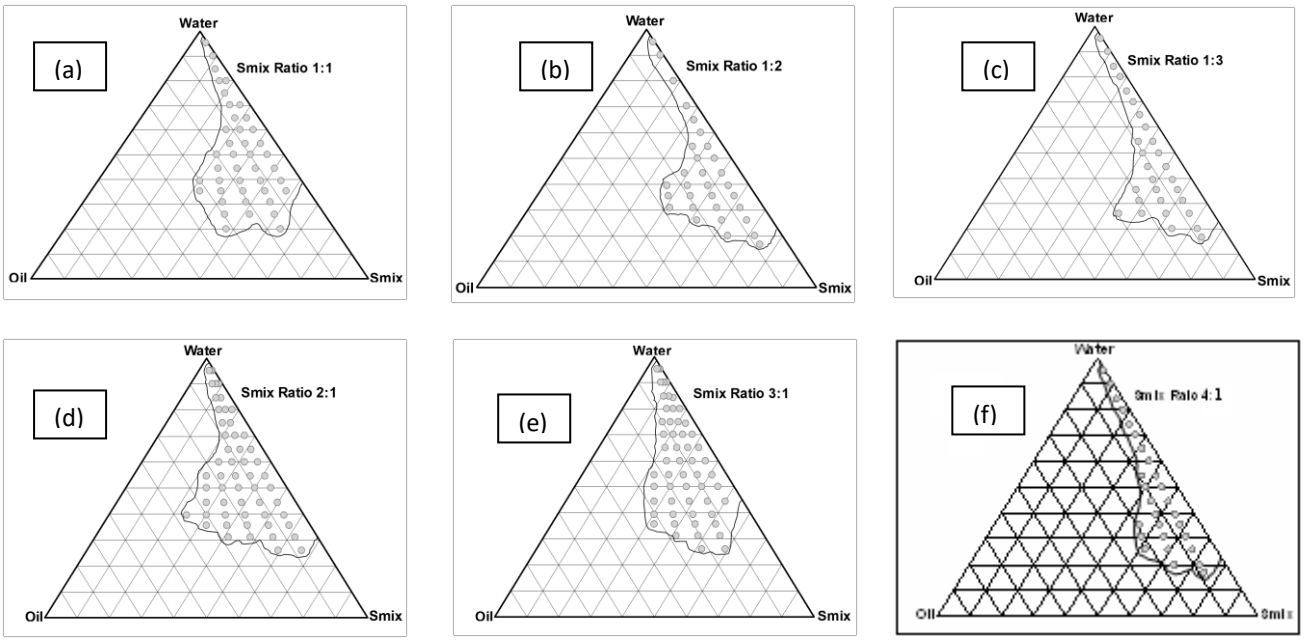

Figure 1: Phase diagrams indicating o/w nanoemulsion (shaded area) at various concentrations of Surfactant (Tween 80) and Cosurfactant (Span 80) i.e. S_{mix} [Fig. 1a (1:1), b(1:2), c(1:3), d(2:1), e(3:1) and f(4:1)].

3.2.Research Design

Existing literature emphasizes the effect of independent parameters, such as Oil to Smix ratio (X1), stirring speed (X2), and stirring time (X3) on the dependent variables viz. globule size and *in vitro* drug release. For statistical optimization, a 2^3 full factorial design was used to optimize and evaluate the mean, quadratic, and interaction effects of these parameters. A total of eight experiments were designed using Design Expert software (version 13), as outlined in Table 4.

Table 4: Results of levels and factors for 2^3 full factorial studies, including statistical descriptors and responses.

Formulation No.	Independent Variables			Dependent Variables	
	Oil: Smix (A)	Stirring Speed (rpm) (B)	Stirring time (Min.) (C)	Globule size (nm)	*In vitro* drug release (%)
1	60	1200	15	27.20	89.67
2	60	1200	20	35.44	84.74
3	60	1000	20	38.49	76.57

4	60	1000	15	32.69	87.64
5	40	1200	15	38.09	79.65
6	40	1000	15	33.93	86.71
7	40	1200	20	36.40	82.2
8	40	1000	20	27.20	89.67

3.3. Optimization of Formulation Variables

3.3.1. Effect of Formulation variables on globule size

The experiments given by the software were used to estimate relationship between independent variables and dependent variables could be represented by quadratic polynomial equation 1. The positive and negative signs indicated the synergistic and antagonistic effect of representative variables in polynomial equation.

$$Globule\ size\ (GS) = +66.38 + 1.36A - 0.7475B - 0.0850C - 0.2250AB + 0.4375AC + 1.70AC$$

The globule size was found to be in a range of 27.2 to 38.49 nm. 3D response surface plot, Figure 2(A & B) showed that variable A i.e. Oil: Smix had synergistic effect on the globule size. At enhanced levels of concentration of A in the formulation lead to increased globule size. Factor B exhibited a slight antagonistic effect on the same. Factor C also imparted a significant antagonistic effect on the globule size of the nano emulsion formulation. Optimized maximum globule size was found to be 32.15 nm at optimum values of all formulation variables.

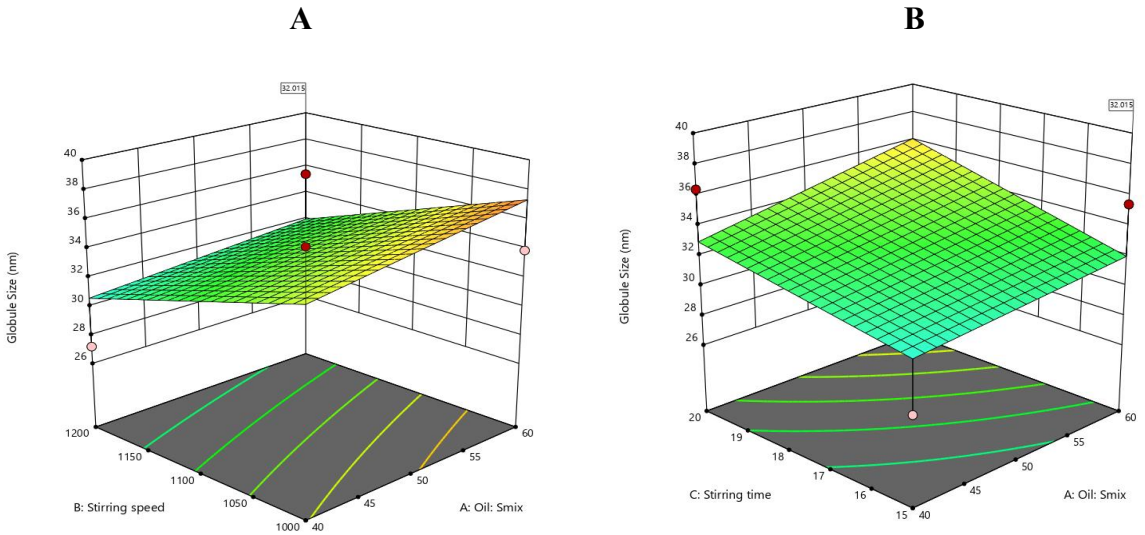

Figure 2: Effect of (A) Oil:Smix and stirring speed (B) Oil: Smix and Stirring time

3.3.2. **Effect of Formulation variables on In-vitro Drug Release**

The relationship between independent variables and *in vitro* drug release is given in equation 2.

$$In\ vitro\ drug\ release\ (IDR) = +84.61 + 0.0787A + 1.46B + 0.1838C + 0.0487AB - 1.22AC - 1.33BC$$

Response surface plots, Figure 3(A & B) showed that all the three factor i.e. A, B and C had very slight effect on the *in vitro* drug release of nano emulsion formulation. Factor A and C had very slight antagonistic effect while factor B had very slight synergistic effect on the same and the maximum value was found to be 88.55% at optimum values of independent variables for optimized nano emulsion formulation.

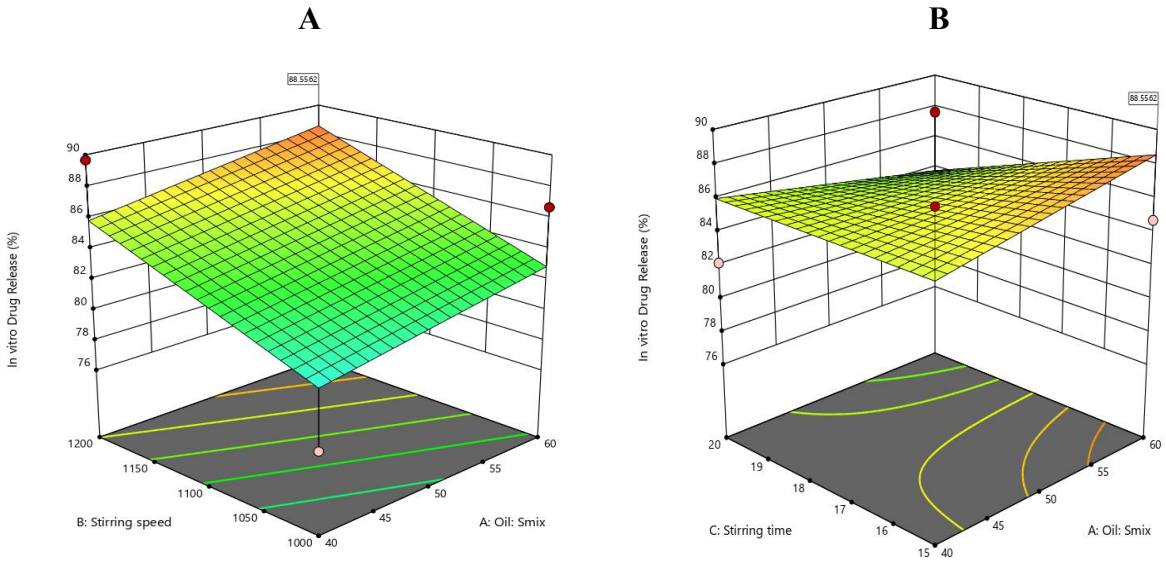

Figure 3: Effect of (A) Oil:Smix and stirring speed (B) Oil: Smix and Stirring time

3.4. Finalization of the optimized formulation

The optimum values of formulation variables were finalized by numerical optimization with maximum desirability of 0.846. High desirability proved that the model was reliable and reasonable [26]. The optimized independent variables were found to be Oil: Smix of 60 w/w, sonication speed of 1200 rpm, and sonication time of 15 minutes.

3.5. Investigations of Thermodynamic Stability

The optimized emulsion underwent various thermodynamic stability assessments, including a centrifugation test, freeze–thaw study, and heat–cooling cycle and results are shown in Table 5. Out of these passed NE formulations at s.no 3 was selected owing to maximum amount of loading of oils and particle size [27].

Table 5: H/C: Heating and cooling (0ºC and 45ºC); Cent: Centrifugation (5000 rpm); Freeze: Freeze-thaw (-21 °C and +25 °C)

Smix	***S. No***	***Formulation variables (%v/v)***				***Observations***			***Inference***
		Oil	S_{mix}	Stirring speed	Stirring Time	H/C	Cent	Freeze	
1:1	1	60	40	1200	15	√	√	√	**Passed**

3.6. Characterization of Nanoemulsion formulation

3.6.1. Zeta Potential, Particle Size, and Polydispersity Index Measurement

These parameters were assessed through dynamic light scattering (DLS) using a Malvern Zeta Sizer (Nano ZS, Malvern Instruments, UK). The measurements revealed a particle size of 225 nm, a PDI of 0.128, and a Zeta Potential of -3.198 Mv indicated the uniform distribution of particles with good stability.

3.6.2. Drug Content (%DC)

It was assessed by isolating the free drug within the nano emulsions through the dialysis method and were found to be 98.33 ± 0.69%.

3.6.3. Study of In-Vitro Drug Release

The drug release from the plain MCL and nano emulsion loaded with MCL was conducted using the dialysis bag method and found that all free MCL was released in just 8 hrs., whereas near about 80% of the MCL was released in 24 hrs represented the sustained release of the formulation as shown in Figure 4 [28].

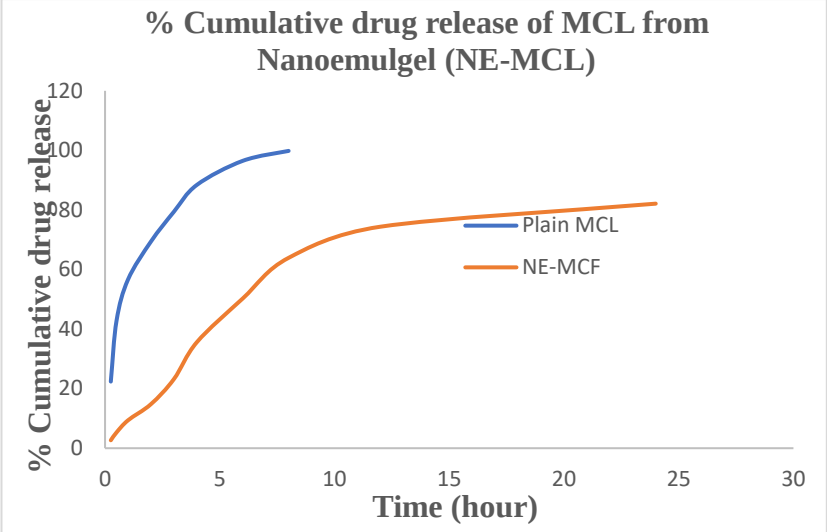

Figure 4: *In vitro* drug release of plain MCL and MCL loaded nanoemulsion formulation

3.6.4. Transmission Electron Microscopy

The morphological examination of NE-MCL was carried out by field-emission scanning electron microscopy using transmission electron microscopy (TEM). The TEM micrographs presented in Figure (5) illustrate spherical globules of pristine quality, smooth texture, devoid of impurities, and falling within the nano size range.

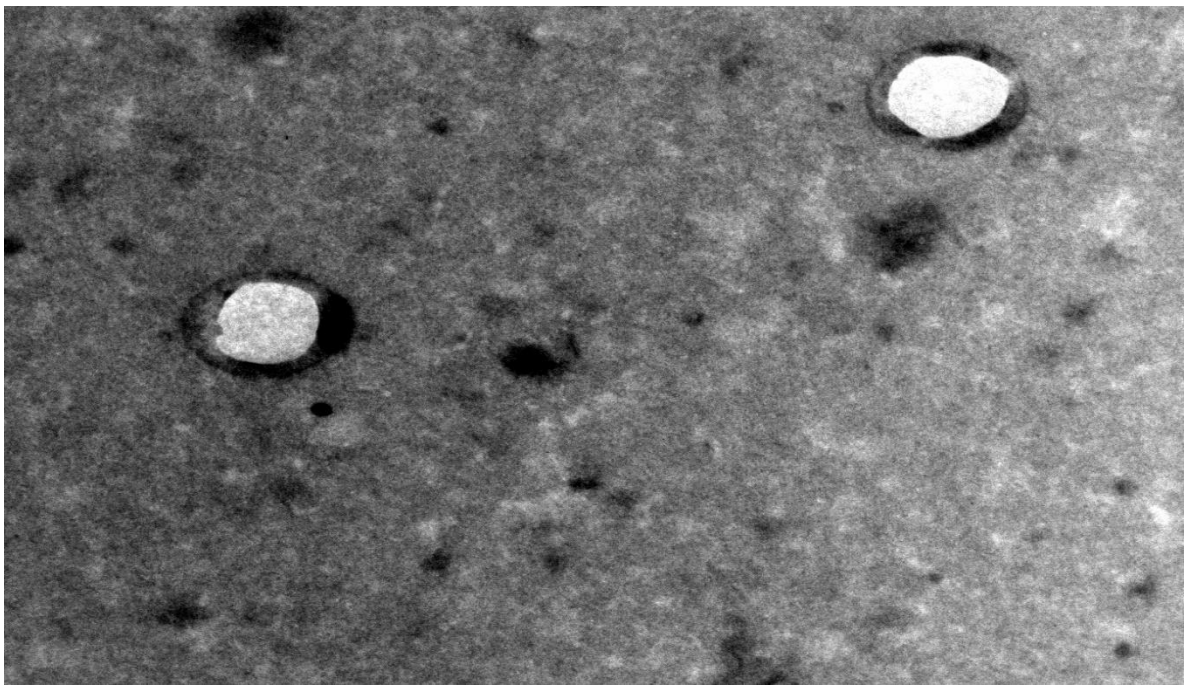

Figure 5: TEM image of drug loaded Nanoemulsion formulation

3.7. Assessment of Nano Emulgel formulation

3.7.2. Determination of pH

The ideal pH range topical formulation was falls around 6.5. Any deviation in the formulation's pH may lead to skin irritation and increased proliferation in bacteria. The pH of the mucoadhesive NE was determined to be 5.78 ± 0.12, ensuring no bacterial growth or tissue irritation attributable to pH variation [29].

3.7.3. Spreadability Analysis

The spreading efficiency of the optimized nanoemulgel was conducted through the graph paper method and were found to be 22.84 cm indicated the good spreading ability of NEG-MCL formulation [30].

3.7.4. Determination of Viscosity

The viscosity of both the plain and mucoadhesive nanoemulsion was assessed by employing a Brookfield viscometer. Elevated viscosity extends the therapeutic duration of formulation at the absorption site by prolonging their contact time and enhancing the drug permeability. The viscosity of the mucoadhesive NE was measured to be 247.10 ± 1.3 cps, representing the optimal viscosity for topical application [31].

3.7.5. *In vitro* release and release Kinetics

The evaluation of the MCL from the nano emulsion (NE) and nanoemulgel formulation was conducted in a Franz diffusion cell using phosphate-buffered saline (PBS, pH 6.4) and depicted in Figure 6. It was found that nanoemulgel formulation decreases the drug release compared to nanoemulsion formulation as gel provide the extra barrier for the drug to be released from. This slow release of drug provides a sustained release for a longer period of time i.e. 24 hrs. The behaviour of the drug release was scrutinized using different mathematical models. The R^2 value for zero order, first order, Higuchi model and Koresmeyer Peppas model was found to be 0.782, 0.815, 0.912 and 0.892 respectively which showed that the drug was release by diffusion-controlled mechanism from the matrix [32].

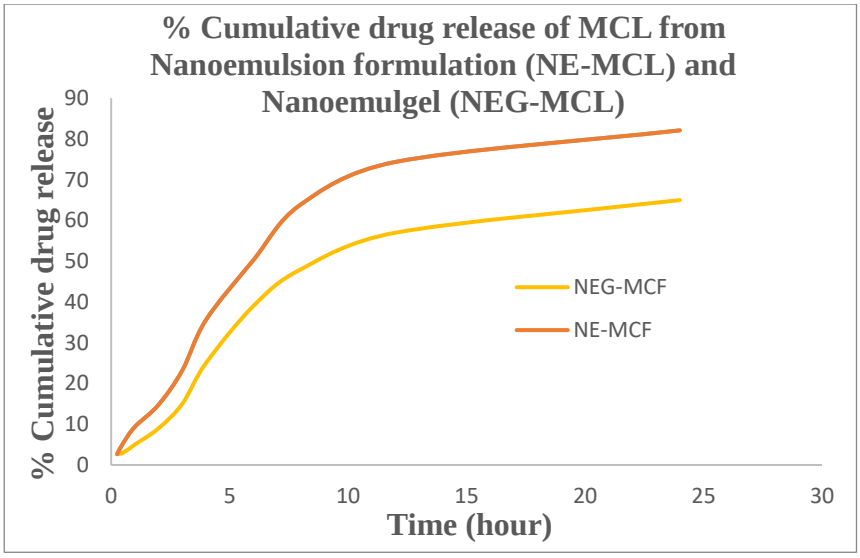

Figure 6: *In vitro* drug release profiles of all the batches

4. Conclusion

The nanoemulgel formulation of methanolic extract of Crinum Latifolium was successfully optimized by phase diagrams and design of experiments for various independent variables and formulated by the hot emulsification method followed by homogenization technique. The optimization of the ratio of surfactant and co-surfactant was carried out using phase diagrams method to formulate a stable emulsion. The formulation was further optimized for the process parameters i.e. Oil: Smix (X1), stirring speed (X2), and stirring time (X3) and found to have a good desirability of 0.846. The stability of the Nanoemulsion was further estimated by the thermodynamic stability. The optimized formulation has uniform particle size distribution and stable zeta potential. The NEG-MCL formulation have a % drug content of 98.33 ± 0.69% with a sustained release effect compared to plain drug. The TEM micrograph showed a perfectly spherical shaped particles in nano size range. The formulation was found to have optimum pH and viscosity with good spradability necessary for topical application. The nanoemulgel formulation showed more sustained effect compared to plain nanoemulsion formulation with a diffusion-controlled drug release mechanism from the matrix. These results showed that nanoemulgel formulation is capable to load a methanolic extract of MCL and can be used in the management of rheumatoid arthritis with the intention of minimizing the systemic side effects of Crinum Latifolium.

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