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Formulation Optimization and Evaluation of Nanoemulsion Loaded with Plant Extract of Crinum latifolium for Antiarthritic Potential

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ABSTRACT

Crinum Latifolium (CL), a plant from the Amaryllidaceae family, exhibits antioxidant, anti-inflammatory, and anti-arthritic properties. This study developed and optimized a nanoemulsion (NE-MCL) formulation of methanolic extract of *C. latifolium* (MCL) for rheumatoid arthritis (RA) treatment, aiming to reduce systemic side effects. MCL was extracted via maceration, and the nanoemulsion was optimized using phase diagrams to determine surfactant and co-surfactant concentrations. Process parameters—oil:Smix ratio, stirring speed, and time—were refined to achieve an optimized NE-MCL with a particle size of 225 nm, polydispersity index of 0.128, zeta potential of -3.198 mV, and drug content of 98.33 ± 0.69%. In vitro studies showed 80% sustained drug release over 24 hours, confirmed by diffusion-controlled kinetics (R^2 = 0.912, Higuchi model). TEM imaging revealed spherical nanoparticles, and NE-MCL demonstrated enhanced release properties compared to the plain drug solution, underscoring its potential to improve RA therapy while minimizing adverse effects.

Keywords: *Optimization, Phase Diagrams, Nanoemulsion, Oral delivery, Rheumatoid arthritis*

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune condition characterized by debilitating joint inflammation, cartilage degradation, and bone loss, significantly impacting patient quality of life [1]. Despite advancements in treatment, current regimens are limited by systemic side effects and suboptimal drug bioavailability [2]. These challenges necessitate the exploration of alternative approaches, including plant-based therapies, which offer multifaceted pharmacological properties and reduced toxicity. *Crinum latifolium* (CL) is a plant renowned for its antioxidant, anti-inflammatory, and anti-arthritic activities, making it a promising candidate for innovative drug delivery systems [3]. Nanoemulsion based formulations have emerged as effective strategies to address the challenges of conventional RA treatments by enhancing the solubility of hydrophobic compounds, ensuring uniform drug distribution, and enabling sustained release [4]. For instance, a study formulated and evaluated a ginger extract-loaded nanoemulgel for the treatment of RA, demonstrating enhanced bioavailability through topical application [5]. This study seeks to amplify the therapeutic potential of methanolic extracts of CL through a nanoemulsion delivery system, aimed at minimizing systemic side effects and improving patient outcomes. Evidence supports the efficacy of plant-based therapies in managing arthritis. For instance, aqueous and ethanolic extracts of *Euphorbia helioscopia* demonstrated significant anti-arthritic potential in adjuvant-induced arthritis models in rats [6]. Similarly, the hydro-alcoholic root extract of *Moringa concanensis* exhibited notable anti-arthritic activity in Complete Freund's Adjuvant-induced arthritis models [7]. Furthermore, ethanolic leaf extracts of *Pisonia grandis* showed dose-dependent anti-arthritic effects, supporting its traditional use as an anti-arthritic agent [8]. These studies highlight the potential of herbal medicine in RA management and underscore the importance of further innovation. *Crinum latifolium*, belonging to the Amaryllidaceae family, is known for its wide range of pharmaceutical activities, including antimicrobial, anti-inflammatory, antitumor, and anticancer properties [9]. Incorporating CL into a nanoemulsion formulation addresses several

limitations of conventional RA treatments, such as poor solubility and limited bioavailability. By leveraging the pharmacological properties of CL, this approach aims to enhance therapeutic efficacy, reduce side effects, and improve patient compliance [10]. Nanoemulsions have shown potential to improve the bioavailability and targeted delivery of bioactive compounds, making them particularly beneficial for chronic conditions like RA. The development of nanoemulsions incorporating plant-based extracts has proven effective in addressing poor solubility and enhancing therapeutic outcomes [11, 12]. The optimized nanoemulsion in this study exhibited improved aqueous solubility and enhanced ex vivo intestinal permeability compared to pure drug suspensions [13]. This underscores the transformative potential of nanoemulsion-based delivery systems in addressing the limitations of poorly water-soluble drugs. By integrating modern nanotechnology with traditional herbal medicine, this research represents a significant advancement in the management of rheumatoid arthritis, paving the way for safer, more effective, and patient-friendly therapeutic options.

2. MATERIALS AND METHODS

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 [14]. 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 [15].

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, Loung 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 [16].

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 [17, 18]. 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 [19]. 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 [20].

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 [21, 22].

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 [23, 24].

iii. Study of *In vitro* Drug Release and Release Kinetics

The *in vitro* drug release study was conducted using the dialysis bag method, involving optimized emulsion and free drug solutions [25, 26]. 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 [27, 28].

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 [29, 30].

e. INVESTIGATIONS OF THERMODYNAMIC STABILITY

The optimized nanoemulsion 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 [31, 32].

3. RESULTS

a. Preliminary Formulation Studies

i. Solubility Analysis in solvents

The solubility of MCF was investigated in different oils and surfactants (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 (Table 3). Thus, used as a surfactant and co-surfactant in the formulation of Nanoemulsion. A 1% Nanoemulsion formulation was prepared based on solubility studies in oil and surfactants.

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 [33].

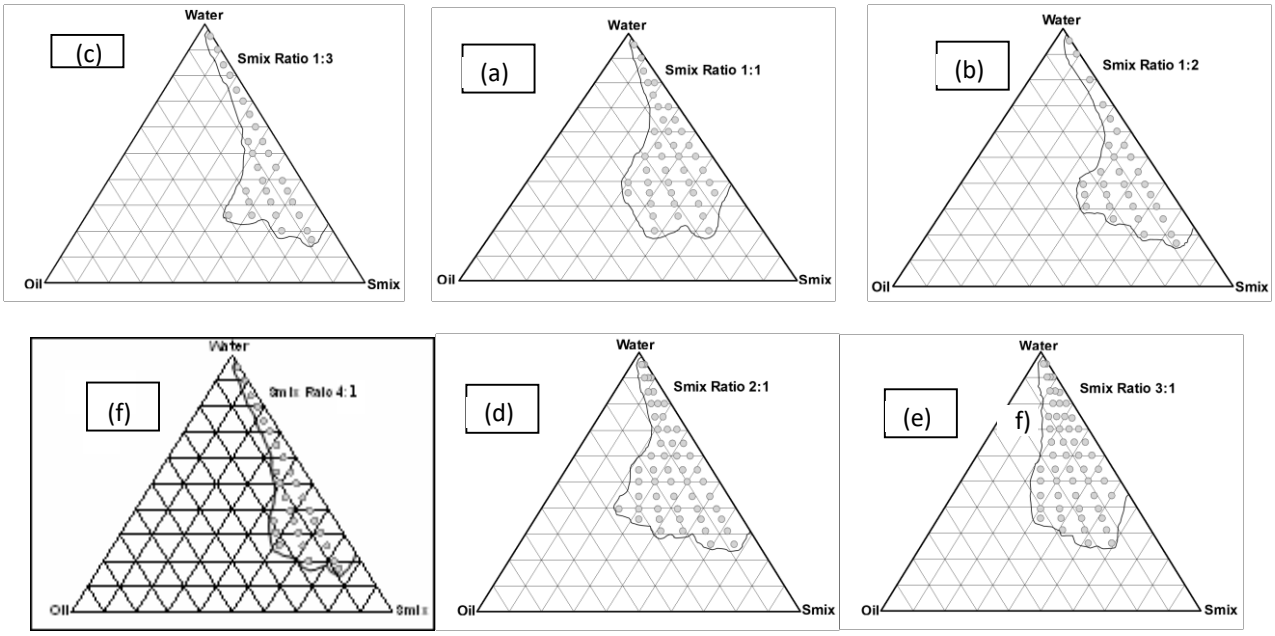

Figure 1: Phase diagrams indicating o/w nanoemulsion (shaded area) at various concentrations of Surfactant (Tween 80) and Co-surfactant (Span 80) i.e. Smix [Fig. 1a (1:1), b (1:2), c (1:3), d (2:1), e (3:1) and f (4:1)].

b. 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	Independent Variables	Dependent Variables

No.	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

c. Optimization of Formulation Variables

i. 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.

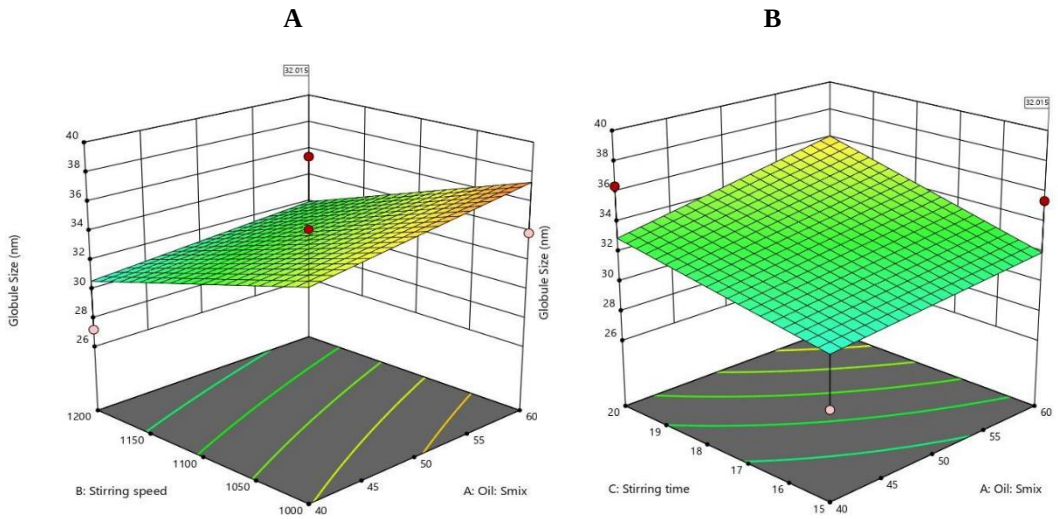

Figure 2: Effect of (A) Oil: Smix and stirring speed (B) Oil: Smix and Stirring timeEffect of Formulation variables on In-vitro Drug Release

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 three factor i.e. A, B and C had a very slight effect on the *in vitro* drug release of nanoemulsion formulation. Factor A and C had a very slight antagonistic effect while factor B had a 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 nanoemulsion formulation.

A **B**

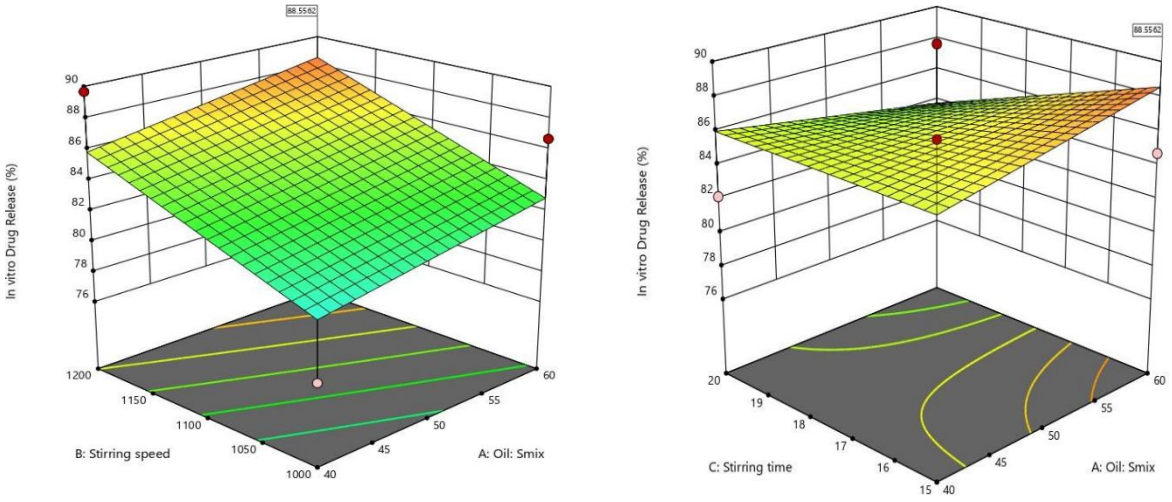

Figure 3: Effect of (A) Oil:Smix and stirring speed (B) Oil: Smix and Stirring time

b. 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 [34]. 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.

c. 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 and found to be stable owing to maximum amount of loading of oils and particle size [35].

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	Smix	Stirring speed	Stirring Time	H/C	Cent	Freeze	
1:1	1	60	40	1200	15	√	√	√	**Passed**

d. Characterization of Nanoemulsion formulation

i. 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.

ii. Drug Content (%DC)

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

iii. Study of *in-vitro* Drug Release

The drug release from the plain MCL and nanoemulsion 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 [36]. The behavior 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 released by diffusion-controlled mechanism from the matrix.

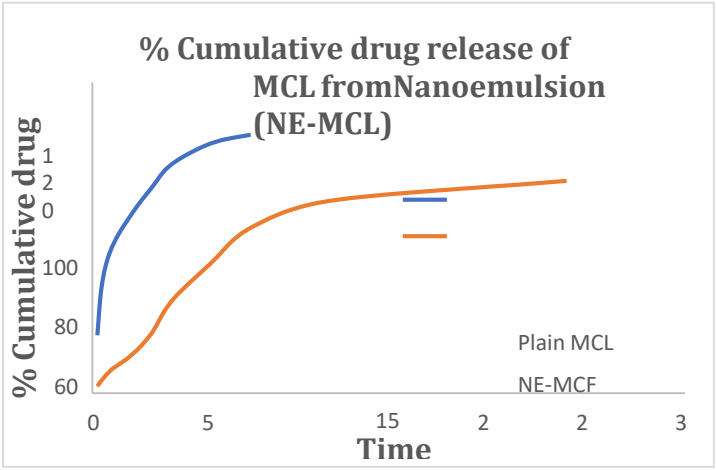

Figure 4: *In vitro* drug release of plain MCL and MCL loaded nanoemulsion formulation

iv. 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 [37, 38].

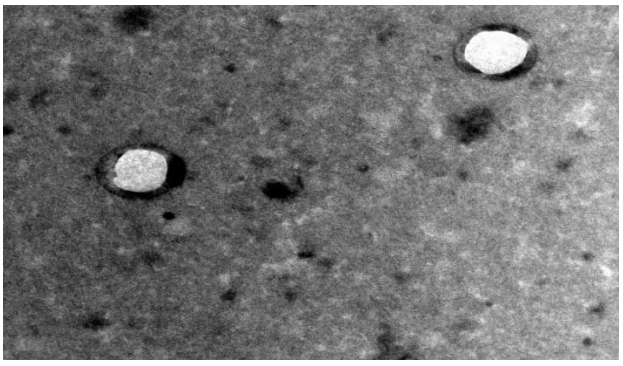

Figure 5: TEM image of drug loaded Nanoemulsion formulation

4. DISCUSSION

The optimized NE-MCL demonstrated a particle size of 225 nm, a low polydispersity index (0.128), and a zeta potential of -3.198 mV, indicating a uniform and stable nanoemulsion system. These characteristics are crucial for achieving consistent drug release, enhanced solubility, and prolonged systemic circulation, which is particularly beneficial in managing chronic conditions like RA. Additionally, the high drug content (98.33 ± 0.69%) underscores the efficiency of the formulation process and its potential for clinical scalability [39].

In vitro drug release studies revealed a sustained release profile, with 80% of MCL released over 24 hours, following a

diffusion-controlled mechanism as confirmed by the high R^2 values of the Higuchi (0.912) and Korsmeyer-Peppas (0.892) models. This sustained release reduces the frequency of drug administration, thereby improving patient compliance and potentially mitigating adverse effects associated with conventional formulations. Furthermore, the spherical morphology of nanoparticles, confirmed through TEM imaging, is consistent with the desired characteristics for enhanced cellular uptake and tissue penetration [40].

Compared to plain MCL solutions, NE-MCL demonstrated superior performance in terms of drug stability and controlled release. This improvement can be attributed to the encapsulation of MCL within the oil phase of the nanoemulsion, which protects the active compound from degradation and facilitates a gradual release into the target site. The thermodynamic stability studies further validated the robustness of the formulation, suggesting its suitability for long-term storage and clinical use.

From a therapeutic perspective, the sustained anti-inflammatory and antioxidant effects of MCL encapsulated in a nanoemulsion could provide a dual benefit in RA management by alleviating inflammation and protecting joint tissues from oxidative damage. The incorporation of C. latifolium into a nanoemulsion delivery system offers a novel and effective approach to harnessing its pharmacological properties while addressing the limitations of traditional RA therapies.

The study also highlights the broader implications of nanoemulsion technology in the field of herbal medicine. The ability to enhance the bioavailability and therapeutic efficacy of plant-based extracts can pave the way for integrating traditional medicine into modern healthcare. Future investigations should focus on in vivo evaluations, exploring pharmacokinetic parameters, and conducting clinical trials to further validate the efficacy and safety of NE-MCL [41].

5. CONCLUSION

The nanoemulsion formulation of methanolic extract of *Crinum latifolium* was successfully formulated by the hot emulsification method followed by homogenization technique. The optimization was carried out by phase diagrams and design of experiments for various independent variables. The optimization of the ratio of surfactant and co-surfactant was carried out using phase diagrams method to formulate a stable nano 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 nano emulsion was further estimated by the thermodynamic stability. The optimized formulation had uniform particle size distribution with good (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 nanoemulsion formulation showed sustained release effect with a diffusion-controlled release mechanism from the matrix. These results showed that nanoemulsion 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* .

6. ACKNOWLEDGEMENT:

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